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THE COURSE OF RECOVERY OF THE SPINAL CORD FROM ASPHYXIA

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(Received for publication October 31, 1938)

INTRODUCTION

THE EFFECT of the onset of asphyxia on the spinal reflexes has been investigated many times; recently by Montgomery and Luckhardt (1929), by King, Garrey, and Bryan (1932) and by Porter, Blair and Bohmfalk (1938). During the development of asphyxia a period of increased reflex activity, which precedes the abolition of the reflexes, has been usually described. In this paper experiments will be described in which the spinal cord of cats has been asphyxiated for various periods; the animals were observed up to three weeks after the experiment, and the recovery of reflex activity after asphyxia was especially studied.

The spinal cord is a difficult organ to asphyxiate by operative methods because it is abundantly supplied with blood vessels. Tureen (1936) found it necessary to clamp the thoracic aorta to obtain a complete anoxemia of the lower part of the spinal cord. He succeeded in keeping only alive cats that had been subjected to this procedure for periods up to 15 min. In our preliminary attempts to asphyxiate the cord such operative methods were tried. The cord itself was ligated in the lower thoracic region to stop the blood supply from the spinal arteries. The aorta was clamped just below the point where the renal arteries branch off, thus preventing damage to the kidneys. The coeliac, superior mesenteric, adreno-lumbar and lumbar arteries, all of which branch off between the aorta clamp and the diaphragm, were clamped also. As an extra precaution the gut was clamped with intestinal forceps. The animal was given ether anaesthesia during the experiment. Many of these animals in which the cord had been asphyxiated for 45–50 min. were observed for several weeks following the operation.

The results in cats subjected to this complicated operation were variable. The prime cause of the variability was probably that the blood vessels to the body muscles anastomose along the whole length of the body and therefore the asphyxia of the cord was often incomplete. The other disadvantage was that the entire posterior half of the body was asphyxiated. This made it difficult to decide whether the symptoms developing after the operation were entirely the result of asphyxia of the cord. The mortality was high. These preliminary experiments agreed in the main with those now to be described.

METHOD

The principle of this second method, used in obtaining the data here reported, was employed by Cushing in 1902. It involves raising the pressure inside the vertebral canal

to a higher level than the blood pressure, thereby occluding the blood vessels supplying its contents. Five days previous to the planned asphyxia the vertebral canal was aseptically opened and the cord ligated at Th9 to Th10, without opening the dura. This made it possible to apply pressure to the lower half of the cord and to avoid compressing the anterior part of the nervous system. The five day period between the operation and the asphyxia allowed the spinal cord to recover from shock. A thick injection needle was inserted intradurally between L6 and L7, and through it sterile Ringer-Locke solution was brought into the subdural space under a pressure of 23–25 cm. Hg. To exclude oxygen from the solution, pure nitrogen was bubbled through it previous to the experiment and was also used to produce and maintain the pressure. Under this pressure the physiological

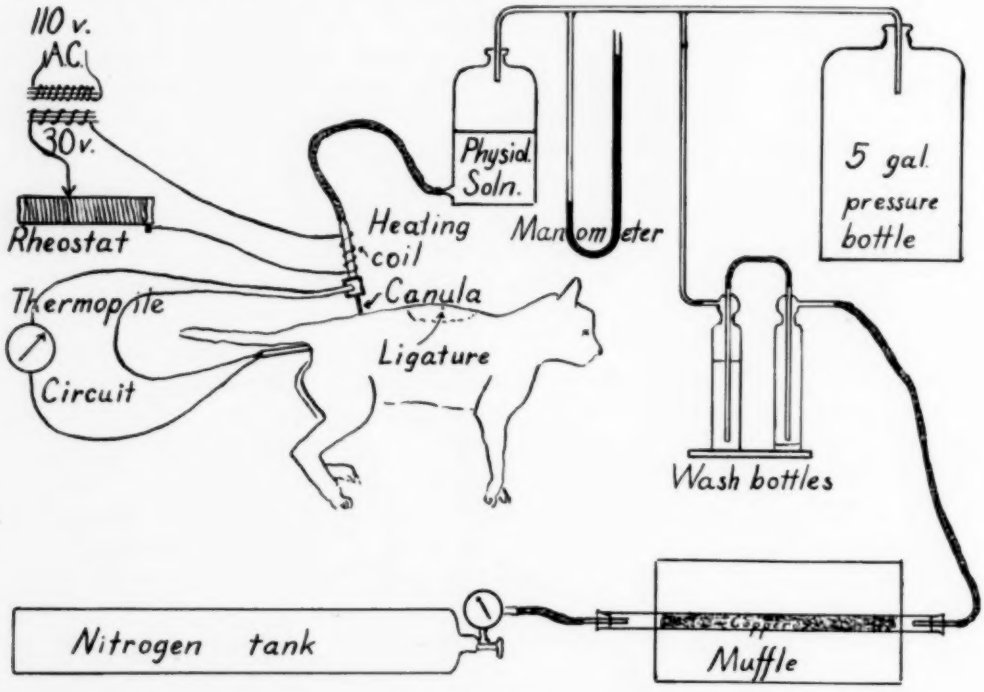

FIG. 1. Diagram of the apparatus used in asphyxiating the cord. See text for a description of the procedure used.

solution disappeared from the vertebral canal at a rate of 1 to 4 cc. per min.; this lost fluid was replaced by physiological solution from a reservoir.

To keep the temperature of the cord from falling, which would depress the metabolism of the cord and introduce a variable factor, the solution was maintained at body temperature by passing it through a tube around which a heating coil was wound. The current through this coil was so adjusted that a galvanometer contained in a thermopile circuit measuring the temperature difference between the solution in the needle and cat's rectum did not show a deflection (Fig. 1). In a number of cases the rectal temperature was measured during the experiment; it was always found to be normal. Fig. 1 is a diagram of the set-up. Controls showed that this pressure method really prevents blood from reaching the lower half of the cord. While the cord was under pressure, 10 cc. of India ink were injected intravenously. The animal was then killed and the cord fixed with formalin. The pressure was maintained in the caudal part of the dural cavity during fixation by changing the physiological solution to formalin under pressure. An abundance of carbon particles was present in the blood vessels of the cord in sections taken cranial to the ligature, none in the caudal cord.

The advantages of this method are obvious. The spinal cord only is asphyxiated, the

peripheral part of the reflex apparatus receiving its normal blood supply, and thus changes in the reflexes can only be ascribed to changes in the central part of the arc. It might be objected that this procedure subjects the spinal cord not only to asphyxia but also to pressure. A small pressure of 23–25 cm. Hg acting equally on every part of the cord does not produce any injury, as demonstrated by the innocuousness of much higher pressure in caissons. The sudden change from high to normal pressure at the inter-vertebral foramina, where the spinal nerves pass, might produce mechanical injury to the spinal nerves, but this is ruled out by the fact that Marchi preparations of the dorsal roots of an animal in which the cord was asphyxiated for 75 min. did not show any degeneration 20 days after the experiment.

Results

The spinal cord was asphyxiated by pressure for periods ranging from 25 to 75 min. Before beginning the experiment the reflexes and tone in the hind legs were tested. They were usually typical for cats made spinal 5 days previously; the knee jerk was brisk, there was a strong flexor reflex with appreciable crossed extension, and the tone was low and usually on the flexor side. The presence of the achilles tendon reflex was variable. When pressure had been applied to the cord, the knee jerk was tested frequently as a measure of the progress of asphyxia. It was usually abolished after 1–1.5 min. of pressure, decidedly quicker than when other methods are used to produce asphyxia. The knee jerk was increased for a short period before disappearing, and in some experiments tonic and clonic hind leg movements were observed shortly after beginning the asphyxia. These movements usually stopped within a minute; in a few cats, however, a slight tonic extensor contraction lasted as long as 10 min.

When the animal was restive, light ether anaesthesia was used only after pressure had been applied and was always discontinued at least 10 min. before the end of the experiment. Often no anaesthetic was necessary, merely stroking the cat sufficing to keep it quiet. The asphyxiated part of the cord was thus never narcotized at any time. After releasing the pressure the return of reflexes and tone in the hind legs was carefully followed several times an hour, then hourly, and, after a few days, once daily. The first sign of returning reflex activity was either a slight tone in the triceps or quadriceps, or a weak achilles tendon reflex or knee jerk. The time required for the return of reflexes and the further behavior of the cord varied with the duration of asphyxia. We shall, therefore, discuss separately each group of animals subjected to a like period of asphyxia.

In two cats pressure was applied for 75 min. Neither animal gave any certain sign of reflex activity in the hind legs during the three weeks of survival. In 5 cats the spinal cord was asphyxiated for 65 min. The times of appearance, and subsequent disappearance, of tone and reflexes are given in Table 1. In one animal (65B) neither tone nor reflexes were observed at any time. In one (65A) which gave tendon reflexes, tone reached a maximum 5 hr. after asphyxia and then declined, both tone and reflexes disappearing completely within 24 hr. The other three cats showed tone only, which appeared some hours after the asphyxia and disappeared completely a few hours later. Tone was observed only in the extensor muscles. After

the disappearance of tone (and reflexes in cat 65A) no reflexes of any kind could be elicited during the period of survival, in some cases as long as three weeks.

The cord was asphyxiated for 55 min. in 5 animals. Tone in the extensor muscles and tendon reflexes returned in all, but only temporarily (see

Table 1. The appearance and disappearance of tone and reflexes in cats in which the spinal cord was asphyxiated. The cords indicated as 25A, 25B etc. were asphyxiated for 25 min.; those indicated as 35A, 35B etc. were asphyxiated for 35 min. etc. An asterisk means that the reflex or tone remained until death.

Cat	Tone Begin (hr.)	Tone End (hr.)	Knee jerk Begin (hr.)	Knee jerk End (hr.)	Ach. t. refl. Begin (hr.)	Ach. t. refl. End (hr.)	Flex. refl. Begin (hr.)	Flex. refl. End (hr.)	Tail refl. Begin (hr.)	Tail refl. End (hr.)	Period of observation (days)
25A	1.5	*	0.5	*	0.5	*	0.5	*	1	*	21
25B	2	*	1	*	1	*	2.5	*	8	*	2
25C	0.3	*	1	*	0.5	*	3.5	*	1.5	*	3
25D	2	*	0.5	*	9	*	6	*	1	*	4
25E	0.3	*	1	*	—		4	*	4	*	20
35A	1	*	0.5	*	0.5	*	9	*	9	*	20
35B	2	*	4	*	1.5	*	24	*	24	*	21
35C	1	*	4	*	4	*	24	*	7	*	18
35D	1.5	*	1	*	1.5	*	7	*	7	*	13
35E	1	72	1	*	1.5	24	—		4.5	*	20
45A	0.2	*	0.2	*	0.3	*	1.5	14	2.5	14	10
45B	1.5	*	0.5	*	1.5	4	—		—		3
45C	6	*	4	*	6	*	—		—		16
45D	2	48	1	48	2	24	10	24	5	48	6
45E	1	32	2	32	2	12	—		7	10	8
55A	2	6	2	5	2	5	—		—		14
55B	2.5	48	2	48	2.5	32	—		—		10
55C	1.5	48	1.5	32	1	24	—		—		8
55D	1.5	32	4.5	48	3	32	—		—		21
55E	1.5	14	8	24	—		—		—		4
65A	4	9	4	24	2.5	24	—		—		2
65B	—		—		—		—		—		7
65C	2	5	—		—		—		—		20
65D	1.5	3.5	—		—		—		—		3
65E	1.5	5.5	—		—		—		—		20

Table 1). The extensor tone became so high in some cats that the tendon reflexes were masked. Before this powerful tone developed the tendon reflexes themselves were quite brisk. Here we encountered a phenomenon that will be met a number of times in this paper: asphyxia caused a marked increase of the reflex excitability of the cord not only at the beginning of asphyxia but for long periods after restoration of the circulation. Another sign of the increased excitability was the clonus observed in these animals either following a knee jerk or achilles tendon reflex or "spontaneously" when the leg was in certain positions. The maximum tonus in cats 55A, B

and E was comparable to that in the fore legs during moderate decerebrate rigidity; that in cat 55C was markedly higher. Neither reflexes nor tone remained longer than 48 hr. after the asphyxia in any case, but after 5 to 6 days a moderate extensor tone redeveloped in three of the cats. In one (55D) even a small but easily elicited knee jerk reappeared. No flexor or tail reflexes were observed at any time after asphyxia.

The next group of 5 animals was subjected to spinal pressure for 45 min. In 2 cats (45D and E) the appearance and disappearance of tone and reflexes was quite similar to that in the 55-minute group, except that a slight flexor reflex and slight movements on pinching the tail appeared temporarily, as noted in Table 1. The other animals differed in that the tendon reflexes and tone remained until death. Indeed, in cats 45A and C, the extensor tone became so great that it could be compared only to a well developed rigor mortis; the extended legs easily supported the weight of the body during the whole survival period (10 and 16 days). In all cases strong tendon reflexes and clonus were observed at some period following asphyxia until they disappeared or became masked by the intense tonus.

FIG. 2. Photograph of cat 35A, in which the spinal cord was asphyxiated for 35 min. (12 days after asphyxia). The animal is standing unassisted; note the extreme stretching of the hind legs which easily carry the body weight.

In the 35-minute asphyxia group of 5 cats (Table 1), the typical spinal reflexes returned and remained (except that the crossed extension reflex was only observed in cat 35B). The tone in the extensor muscles usually became very high. Fig. 2 is a photograph of cat 35A showing that the strongly extended hind legs can easily carry the body weight. In some animals the strong extensor tone was present during the first two days after asphyxia and then disappeared (35D and E); in others it was only moderate for the first two days, but increased greatly after that (35A and B), and remained during the period of observation, 20 and 21 days. In one animal, the tone did not become high at any time (35C). Vigorous tendon reflexes and clonus were observed in all cases. In cat 35C the tail reflexes became so extremely brisk that the slightest touch of the hair would evoke movements.

Pressure was applied for 25 min. to the final group of 5 animals (Table 1). Tone and tendon reflexes returned between 20 min. and 2 hr. Tail reflexes and the flexion reflex, usually with crossed extension, developed in all 5 animals within 9 hr. The tone was slight to moderate, except that in cat 25E it became intense after the first two days and remained so until after the

seventh day. Cat 25B developed a preponderant and continuous flexor tone and cat 25D extremely brisk tail reflexes. These animals resembled normal spinal cats in many respects, though their reflexes were more vivid.

The increase of reflex excitability and tone after asphyxia

Asphyxia of the cord thus commonly resulted in increased reflex excitability and high extensor tone. After an asphyxia of 35 to 55 min. exaggerated tendon reflexes were usually present and could be evoked by the slightest tap on the tendon so long as excessive tone in the extensor muscles did not mask them. The tendon achilles reflex offered a convincing example of this heightened response. Five days after cord ligation this reflex was usually low and sometimes absent. After asphyxia it was often the first reflex to return and usually became strong after some hours. The marked clonus in the extensor muscles frequently observed after asphyxia is another sign of the increased excitability. The skin reflexes were seldom marked, but very readily elicited tail reflexes were observed in one 25-minute and one 35-minute cat.

The question arises whether the high extensor tone is the result of a real reflex or whether the motor neurons discharge spontaneously. There is some reason to believe that the latter event could occur. Fibrillar contractions of the toes and muscles of the leg were often seen for a few hours after asphyxia; this type of contraction is usually considered evidence of damage to the anterior horn cells. These fibrillations always disappeared shortly, however, while the tone could continue for weeks.

Direct proof that the tone was of reflex origin was provided by its decrease after dorsal root section. The cord was carefully exposed in 7 animals showing marked tone and the dorsal roots transected on one side from L5 down. The following animals were used: 55-minute group, cat 55D after 21 days; 45-minute group, cat 55A after 10 days, and a special animal, not included in the table, which developed a high tone in 6 hours when the roots were cut; 35-minute group, cats 35A, C and E after 23, 18 and 20 days respectively; 25-minute group, cat 25E after 20 days. The result was always that the tone on the transected side either disappeared or diminished greatly; on the opposite side the tone remained. As in decerebrate rigidity, the post-asphyxial tone in the extensor muscles can persist for days and even weeks and it is located in the extensor muscles of the large joints with the toe muscles not participating.

The time course of tonus changes falls into three categories. In type A, seen after 35 to 65 min. asphyxia, tone appears a few hours after asphyxia, sometimes reaches a considerable height, and disappears again within 48 hr. Five to six days later slight tone may develop again. In type B, after 25 to 45 min. asphyxia, the tone is rather low for the first 2 days and then increases to a considerable height (comparable to rigor mortis). In type C, after 25 min. of asphyxia and in one case after 35, the tone remains low throughout.

Tureen (1936) also observed periods of high tone and increased reflex excitability in cats after short periods of asphyxia.

Histological changes

The lower spinal cords, level L6 to L7, from cats that survived the asphyxia 14 days or longer and from normals were fixed in 95 per cent alcohol, dehydrated, embedded in paraffin, sectioned and stained with toluidine blue. Even asphyxia of 25 min. caused a marked decrease in the number of nerve

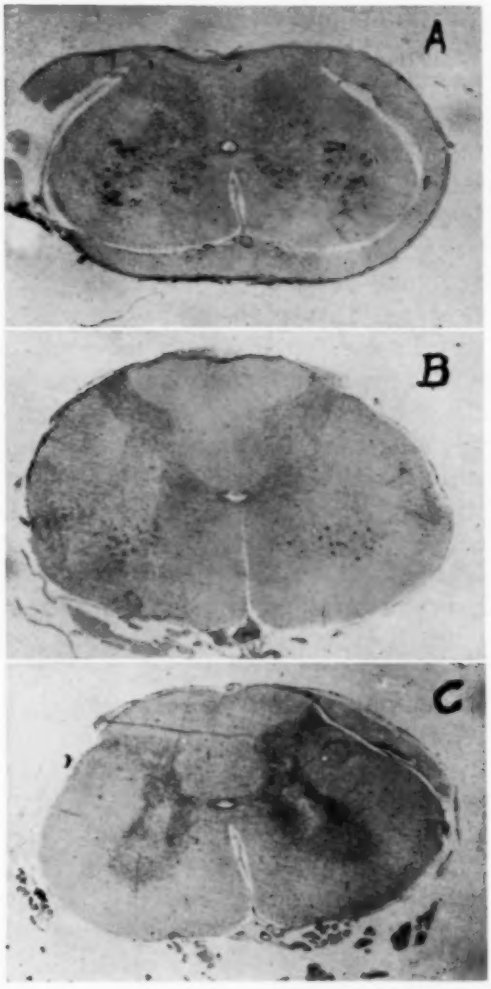

FIG. 3. Photomicrographs of sections of the spinal cord taken at L6. The sections were stained with toluidine blue and photographed through a red filter. A, normal cord. Notice the numerous larger anterior horn cells and the great number of smaller ganglion cells in the grey matter. B, preparation of cat 25E, taken about 3 weeks after a 25-minute asphyxia of the cord. The anterior horn cells are less numerous. Remarkable, however, is the diminution in the number of the smaller ganglion cells. In this animal a high tone was temporarily observed. C, preparation of the cord of cat 65C, subjected to asphyxia for 65 min. 3 weeks previously. Note the absence of any cell bodies.

cells, as compared with the normal, although a fair number of large motor cells remained in the anterior horn. The smaller ganglion cells in the rest of the grey substance suffered more than the large motor neurons (Fig. 3). After an asphyxia of 45 min. only a few anterior horn cells were left and the smaller ganglion cells had almost disappeared. After 55 and 65 min. only an occasional cell could be found. In many preparations the large ganglion cells in the anterior horn were counted. Average values are: normal, 33; 25-minute asphyxia, 25; 35-minute, 11; 45-minute, 6; 55-minute, 2; and after 65-minute, only 1.

Discussion

Certain durations of asphyxia caused a reappearance of reflexes and tone which was only temporary, lasting less than 48 hr. Since tone and even tendon reflexes have been seen to reappear temporarily after cord asphyxia of 65 min. and since subsequent histological preparations from these animals show almost every ganglion cell killed, it must be concluded that neurons which have been so greatly damaged that they will shortly die are still able to function temporarily, with full reflex conduction maintained, including synaptic transmission. The return of tone in three 55-minute cats, and of tendon reflexes in one of these, 5 to 6 days after an initial temporary presence indicates that after this asphyxia the perikarya could not have been completely destroyed. But it may be assumed that the neurons were damaged to such an extent that certain functions required for the maintenance of conduction were temporarily abolished, to return 5 to 6 days later. Since the tone developing 5 to 6 days after asphyxia was slight, presumably a large majority of the cells was killed. After shorter asphyxia a larger number of cells survive and must maintain continuous function.

There is an increasing resistance of the reflex mechanism from skin reflexes to tendon reflexes to tone. Four of five 65-minute cats showed temporarily an appreciable tone; tendon reflexes reappeared regularly in the 55-minute animals and in one 65-minute cat. Skin reflexes reappeared regularly after 35 min. of asphyxia and twice in the 45-minute animals. This difference in resistance may be explained by differences in sensitivity of the neurons or in the number of neurons involved in the several reflex arcs. With fewer neurons in the arc the less is the chance of one dropping out, and in this respect it is significant that tendon (and tone) reflexes are supposed to have the most simple reflex arcs.

The persistence of decerebrate rigidity has caused it to be considered a release phenomenon (Bazett and Penfield, 1922). The increased tone which may persist for weeks after asphyxia might similarly be the consequence of a release. This would mean that a functional system which normally inhibits the reflex activity of the spinal cord is abolished by asphyxia, releasing the excitatory activity of the cord. It may well be a general rule that the inhibiting systems of the central nervous system are more sensitive to asphyxia than the excitatory ones, as Barcroft and Barron (1937) found for sheep embryos. The tone in a spinal animal would, then, be interpreted as an equilibrium between excitatory and inhibitory components. Asphyxia, by damaging the inhibitory component more severely than the excitatory one, shifts the equilibrium towards the excitatory side. We have further evidence that a tone inhibiting system is damaged following asphyxia. In some cases, forcible bending of a hind leg having high extensor tone was followed by a spring-like return upon release; in others by the disappearance of tone for several seconds (lengthening reaction). In these latter cases the inhibiting component was still capable of function, whereas in the former it was abolished.

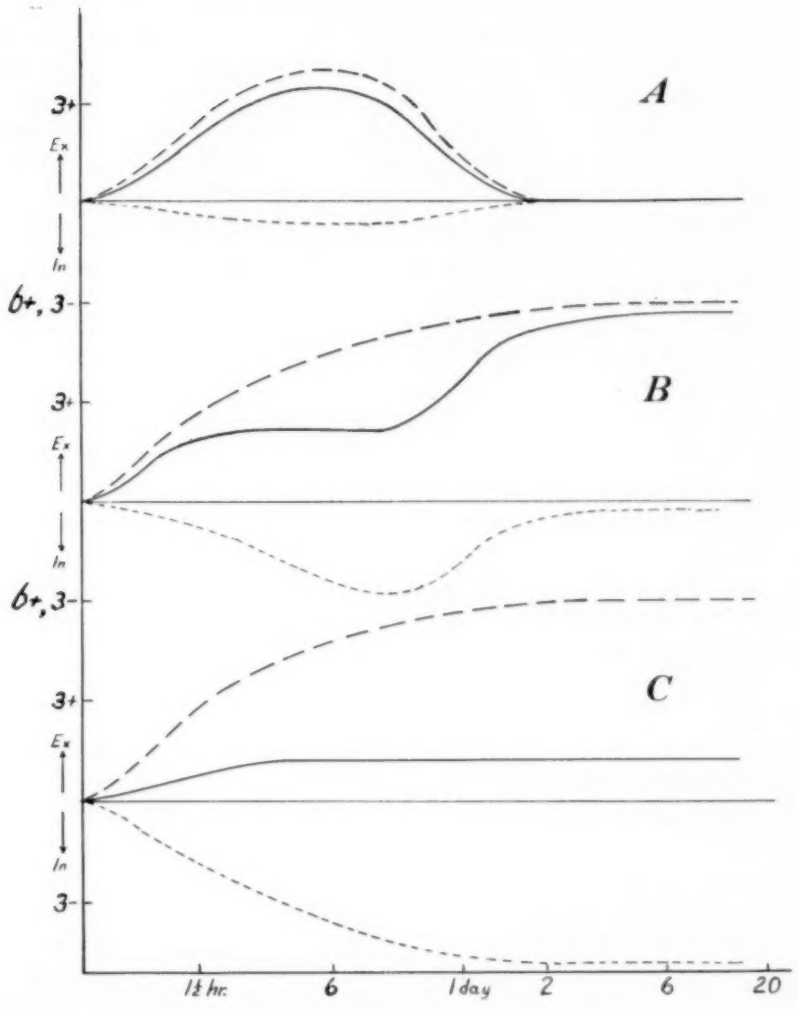

FIG. 4. Diagrammatic explanation of the course of tone in the extensors of the hind leg after asphyxia designated in the text as types A, B, and C. The dashed curves above each horizontal axis represent the activity of the structures involved in the excitatory component of the reflex causing the extensor tone; the dotted curves below the axis represent the activity of the structures involved in the inhibitory component of this reflex; and the algebraic sum of these two curves, representing the actual tone, is the full line appearing above each axis. This full curve corresponds to the tone usually found in animals whose cords have been asphyxiated: A, 65 and 55 min.; B, 35 and 45 min.; C, 25 min. The positive ordinates represent an arbitrary scale of tone: from 0 to 3, tone progresses from absolute flaccidity to that seen in a moderate decerebrate rigidity of the front legs; from this point to 6 it increases until it is comparable to rigor mortis. The abscissae represent the time on a logarithmic scale.

An explanation of the three types of effect of asphyxia on extensor tone is possible along these lines (Fig. 4). After long asphyxia tone reappears only temporarily, after shorter periods it remains. Presumably after long as-

phyxia the excitatory component functions temporarily, after shorter periods permanently; and the same may be assumed for the inhibitory component. This latter component has less resistance to asphyxia. A certain period of asphyxia, then, might damage the inhibitory component to such an extent that it resumes function only temporarily, while the excitatory component will remain permanently. We believe this to be the explanation of type B curve, observed after asphyxia for 25 to 45 min. For 2 days both components of the tone reflex function, resulting in a moderate tone; later the inhibitory component is lost and the more resistant excitatory component remains unopposed. After the shorter periods of asphyxia both the excitatory and inhibitory components resume their function and a moderate permanent tone results from this equilibrium (type C tone). With sufficient asphyxia to permit the excitatory component only temporary function, the inhibitory element would be severely damaged, leaving excitation more or less unopposed. Actually some of the 55-minute cats did manifest strong extensor tone (type A). After still longer asphyxia (65 min.) extensor tone remains moderate due to damage of the excitatory component. We are aware that the course of tone may be complicated by the recovery of damaged neurons 5 to 6 days after the asphyxia. In cat 25E, asphyxiated for 25 min., the drop of the high tone 7 days after the pressure may have been due to a similar recovery of the inhibiting component.

The explanation given for the high tone after asphyxia would apply also to the increased reflex excitability of the tendon reflexes. Since exaggerated tail reflexes have been observed after asphyxia, it seems that here also a normal inhibiting component was selectively damaged. The excitatory component of these skin reflexes is almost certainly more complicated than in the case of the tendon reflexes, so that the excitatory and inhibitory components probably have a more equal resistance to asphyxia and the selective abolition of the inhibitory component would be less certain. This is in keeping with the observation of Porter (1912), who did not find increased excitability of the flexor reflex during the development of asphyxia. In recent experiments, with more refined methods, however, this has been obtained in a comparable reflex (Porter, Blair, and Bohmfalk, 1938). On this interpretation the increased reflex excitability of the tendon reflexes and perhaps the convulsions which are observed during the development of asphyxia of the cord are to be explained in the same way; namely, by the differential injury of inhibiting systems. The increased reflex excitability observed during the development of asphyxia is not an excitation phenomenon, as often maintained, but, on the contrary, one of release.

SUMMARY

1. The effect of asphyxia on the lumbo-sacral cord of spinal cats was investigated, the asphyxia being produced by raising the intradural pressure above that of the arterial blood, thereby preventing blood from reaching the region involved. Asphyxia was maintained for periods of 25–75 min. and the

behavior of spinal reflexes observed during this time and for as long as 3 weeks following.

2. After long asphyxia (55 and 65 min.) tendon reflexes and tone returned for 48 hr. at the most, then disappeared. In 3 of the 55-minute cats a moderate extensor tone returned again after 5 to 6 days. After shorter periods (35 to 55 min.) these reflexes and the extensor tone returned permanently and were intense. Moderate tone and all the usual reflexes returned shortly after 25 min. of asphyxia and remained.

3. The strong extensor tone is of reflex origin since transection of the dorsal roots of the lower cord abolishes it.

4. Histological preparations of the cord showed that only 3 to 75 per cent of the normal number of anterior horn cells were present 14 days following the asphyxia. The number surviving diminished with increasing duration of asphyxia.

5. It is concluded that the increased reflex excitability and the exaggerated tone are the result of release, the normal inhibitory systems of the cord being more damaged by asphyxia than the excitatory systems.

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CORTICAL RESPONSE TO SENSORY STIMULATION UNDER DEEP BARBITURATE NARCOSIS

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IN A PREVIOUS PAPER (Derbyshire, Rempel, Forbes and Lambert, 1936) a remarkable effect was reported in the deeper stages of avertin and pentobarbital-sodium anesthesia, and in asphyxia*—an effect which differentiated the action of these agents sharply from that of ether. This was a conspicuous electric response of the cerebral cortex to stimulation of the sciatic nerve, appearing with a latency of about 40 to 80 msec. and lasting for a similar or somewhat longer period. It was found only when the cerebrum was so deeply narcotized that the record of its electrical activity afforded a relatively smooth base line as compared with that in the lighter stages of narcosis. It was subject to extremely rapid fatigue (or inhibition) since, when a rapid series of stimuli (5 or more per sec.) was applied to the sciatic nerve, this large wave, or "spike," appeared only in response to the first of the series; a rest of about 0.5 sec. was required to enable another stimulus to evoke the effect, and considerably more than a second if the effect were to attain its initial magnitude. When narcosis was so deep that the base line of the electrical record became quite smooth, it could be seen that the large excursion described above was regularly preceded by a smaller excursion whose latency (from the sciatic stimulus) was about 10 or 12 msec. The agreement of this latency with that of responses recorded in the brain-stem in previous researches (Forbes and Miller, 1922; Leese and Einarson, 1934) led to the inference that this preliminary excursion represents the arrival of the volley of afferent impulses in the cerebrum.

In the experiments of Derbyshire, Rempel, Forbes and Lambert, when the "active" (grid) lead was placed on the sensory or motor area for the hind leg and the ground lead on a cauterized area of the opposite cortex, the large "secondary" spike was regularly positive, *i.e.*, grid electrically positive to ground. In other parts of the cortex, remote from the sensorimotor area, responses of similar time relations were found, in some places grid-negative, in others diphasic. They seem to denote a widespread disturbance. In view of the regular occurrence of this response at a distinct time interval after the arrival of the afferent volley, we propose to designate it the "secondary discharge."

That in moderate narcosis no such effect appears—indeed no recognizable response to sciatic stimulation—whereas when narcosis is so deep that the cortex has become perfectly quiescent this marked response follows the

* In a few experiments performed since the publication of that paper, the same effect has been found under Dial anesthesia. We wish to acknowledge the generous donation of Dial by Ciba Pharmaceutical Products, Inc.

stimulus with almost unfailing regularity, seems paradoxical. That no such effect was found in deep ether anesthesia suggested the conclusion that ether anesthesia when deep blocks the afferent paths leading to the cortex, whereas the hypnotic drugs (pentobarbital and avertin) stop the spontaneous activity of the cortex, yet leave it still accessible to afferent impulses. The secondary discharge thus presents interesting problems. What is its essential nature? What structures are involved, and how is activity distributed among them? To throw some light on these questions we performed a series of experiments of which this is a preliminary report.

METHOD

Cats were used in all experiments. In most of them pentobarbital sodium (nembutal) was the only anesthetic. In some cases dial was used, and in some ether was superposed on the pre-existing pentobarbital anesthesia. Our procedure falls in two main groups: examination of the distribution of the electrical effects in different parts of the cortex, and comparison of the latencies of their various components with those which could be measured in the afferent pathways leading to the cerebral cortex. Sometimes both sciatic nerves were stimulating in the course of a single experiment; sometimes only one. In all cases stimulating electrodes like those used by Derbyshire, Rempel, Forbes and Lambert (1936) were secured in contact with the nerve throughout the experiment. For stimuli, single induction shocks were delivered from a Harvard inductorium by means of a hand-operated mercury-contact key. The strength of stimulation was maximal or nearly so for the flexion reflex.* Time of stimulation was recorded on the film by a small string galvanometer throwing its shadow on the margin of the film (see Forbes and Cattell, 1924).

Recording was done with a Hindle string galvanometer and a direct-coupled amplifier (Forbes and Grass, 1937). The film was run at a high enough speed to facilitate the measurement of latency. After the response had been recorded from the intact cerebral cortex, various extirpations were performed and their effects on the response were observed. Often in a single experiment, several successive portions of one or both cerebral hemispheres were removed, and responses were recorded after each extirpation. Various types of lead-off electrodes were used. In nearly all cases they were of the Ag-AgCl type. In some experiments they were in the form of plates of several sq. mm. in area. More often they were of wire presenting a surface of much less than a square millimeter. Often two such wires were cemented together as a "bipolar" pair. In one significant experiment one wire of each pair projected about 2 mm. beyond the other and could thus be inserted into a layer of active cells while its mate rested on the surface of the cortex. A similar method of leading off was sometimes achieved with a pair of concentric electrodes, as described by Beecher, McDonough and Forbes (1938). In a few experiments a metal plug was screwed into the cranium and through a hole drilled in the plug a needle was inserted until it touched the dura.

In several experiments comparisons were made of different types and arrangements of electrodes for leading off the cortical potentials. Three arrangements were directly compared in a number of records in each of three successive experiments. These three were as follows: (i) Plates applied, as in the experiments of Derbyshire, Rempel, Forbes, and Lambert (1936), one to the dura over the intact cortex, the other (ground lead) to a cauterized area on the opposite cortex. (ii) A plug screwed into the cranium was the ground lead; the needle (grid lead) penetrated the dura and about 0.5 mm. into cortical gray matter. (iii) The needle which passed through the plug, as grid lead; the plate on the cauterized area, as ground. In all three experiments lead ii (needle-plug) regularly gave the largest excursions in the recording system, their voltages amounting often to over 500 μV., or about double the average voltage obtained with either of the other two arrangements (Fig. 1). In one of these experiments the gain in the amplifier was adjusted to compensate for this difference; the resulting comparison showed no difference in wave-form between the two first-named methods of recording.

* Observation of the action current in the sciatic nerve with a cathode ray oscillograph indicated that the stimuli used were well above maximal for A fibers, about threshold for B fibers and probably were usually below threshold for C fibers.

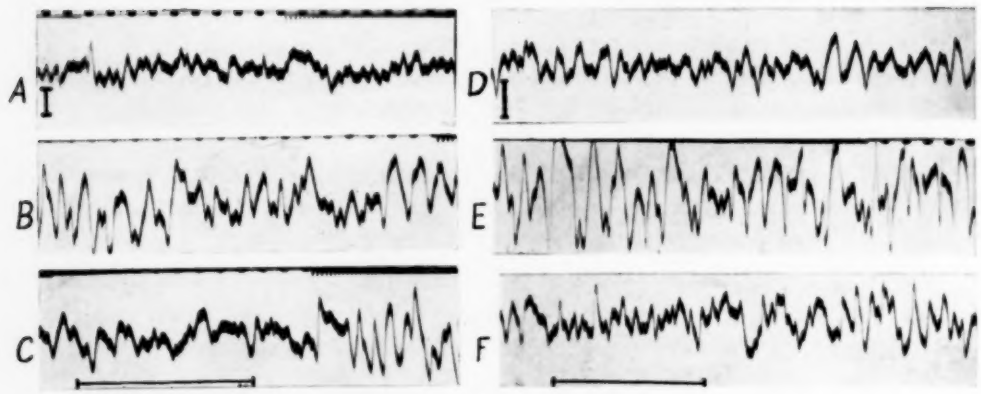

FIG. 1. Activity from cat's sensorimotor cortex under moderate pentobarbital-sodium anesthesia, recorded with different electrode combinations. A, B, C, one experiment; D, E, F, another. A, D: two plates, grid on active cortex, ground on cauterized area. B, E: concentric electrodes with wire to grid, plug (on skull) to ground. C, F: wire to grid, plate on cauterized area to ground. *String galvanometer*. One sec. shown by horizontal lines underneath records. Vertical line beside records represents 200 μV. In each series the amplification was kept constant throughout. In this and in all subsequent records the upward deflection of the string signifies grid negative.

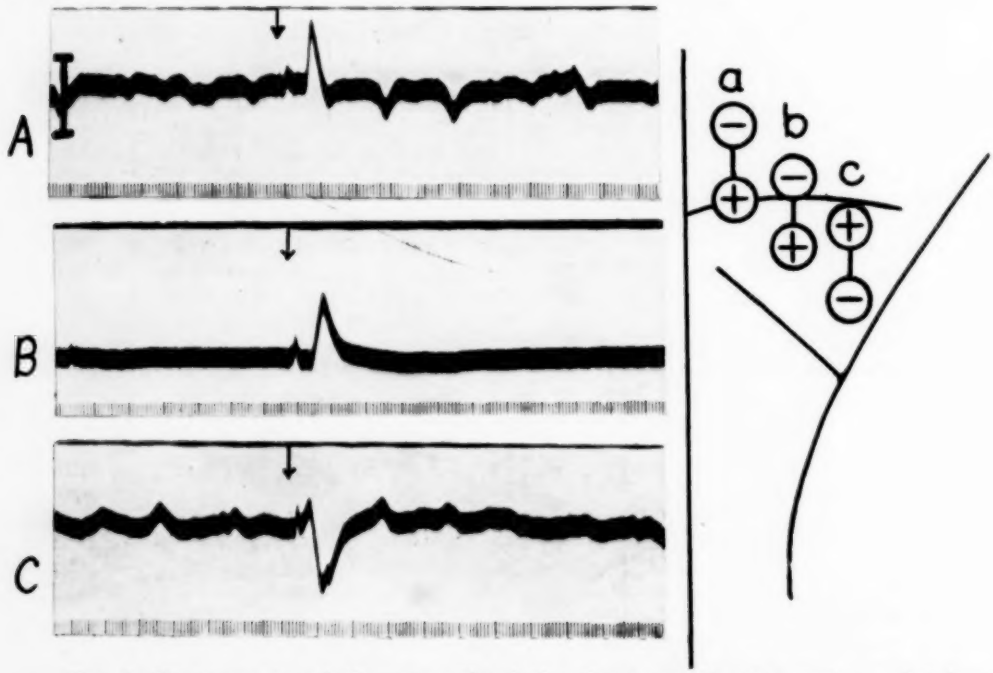

FIG. 2. Responses to sciatic-nerve stimulation from sensorimotor cortex under deep pentobarbital sodium anesthesia, showing both primary and secondary discharges. Diagram shows position of electrodes in these three cases, indicating distribution of positive and negative potentials. Bipolar electrodes. Anterior electrode connected to grid. *Time* (0.01 sec.) shown below records. Vertical line beside records represents 100 μV. Time of stimulation (single shocks) shown by arrow and also by small initial excursions due to electric artifact.

In several experiments we applied a pair of "bipolar" electrodes (fine silver wire, 0.5 to 2 mm. apart), resting on the cortex. Although, in most cases, the spontaneous potentials and secondary discharge thus recorded were much smaller than with the other methods, in some experiments they were as large as with the other types of electrodes (500 μV. or more). This is somewhat surprising, since it suggests a steep potential gradient between adjacent points in areas of the cortex which might be expected to be affected in like manner by afferent volleys and therefore to remain nearly equipotential with respect to one another. It was feared that the apparatus might not be recording a true difference of potential between the leads on the cortex, but rather a transient difference of potential between the grid electrode and the ground, in which the capacity to earth of the animal served as the return lead. This was controlled by reversing the lead wires without disturbing the contacts of the bipolar pair of electrodes with the brain. The resulting reversal of excursions in the galvanometer showed that we were indeed recording a difference of potential between nearby points on the cortex. These bipolar leads were also used to explore

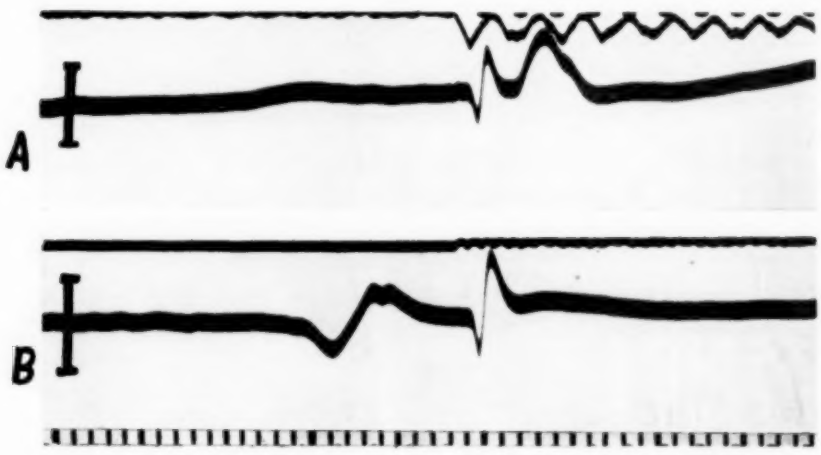

FIG. 3. Effect of recent spontaneous activity on secondary discharge under deep pentobarbital sodium anesthesia. Primary response unaffected. Electrodes: grid, wick, on sensorimotor cortex; ground, plate on skull. In B spontaneous wave precludes secondary discharge. *Time* (0.01 sec.) shown below records. Vertical line beside records represents 500 μV. Time of stimulation (single shocks) shown by initial excursion of signal line on top.

the various parts of the cortex. In one experiment there was a systematic change in the polarity of the secondary discharge as the leads passed a certain critical point. This is illustrated in Fig. 2, in which the accompanying diagram shows where an area became positive during the discharge with respect to adjacent areas both behind and in front of it. We have not explored the cortex enough to make further statement about this distribution.* It seemed probable that a more significant arrangement of leads was from interior to surface, a view supported by the larger voltages obtained when the grid lead penetrated the gray matter (see above). The concentric electrodes described by Beecher, McDonough and Forbes (1938), in which the grid lead penetrates about 2 mm. into the cortex, are perhaps the most satisfactory.

RESULTS

Forbes, Renshaw and Rempel (1937) described isolated spontaneous discharges of the cortex in the deeper stages of pentobarbital narcosis. In

* It is of interest to note that in A and C of Fig. 2 the spontaneous waves in each case are of opposite sign to the secondary discharge.

many cases the excursions in the records were almost identical in form and time relations with the secondary discharge with which we are now dealing. Our experiments have shown that either spontaneous discharges or those evoked by afferent stimulation will for a time prevent the occurrence of a secondary discharge in response to a stimulus. This is illustrated in Fig. 3, which contrasts the response of the inactive cortex with its failure to respond to a stimulus applied shortly after a spontaneous discharge, and in Fig. 4, which shows the effect of repeated stimuli. Many instances of both effects were found and they suggest a long refractory phase in some mechanism involved in the production of both the spontaneous discharges and those induced by stimulation. Our experiments confirm and further emphasize the distinction already drawn between the initial excursion, taken to mark the arrival of the afferent volley in the cerebrum, and the later excursion which we have designated the secondary discharge (cf. Bartley and Heinbecker, 1938). The distinction rests on several different kinds of evidence. Besides the regular difference in latency, already noted, we find a pronounced difference in their behavior on repeated stimulation. As stated above, the secondary discharge fades out completely on repetition of the afferent stimuli at frequencies above about 4 per sec. The initial excursion, which we shall call the primary response, is subject to a slight degree of fatigue (or "equilibration") similar to that reported by Forbes and Miller (1922) in the response of the brain-stem in the decerebrate animal. Typically these excursions, when evoked by stimuli repeated at 5 to 7 per sec., decline to between 50 and 80 per cent of the initial value obtained after a period of rest. Examples of this are shown in Fig. 4.

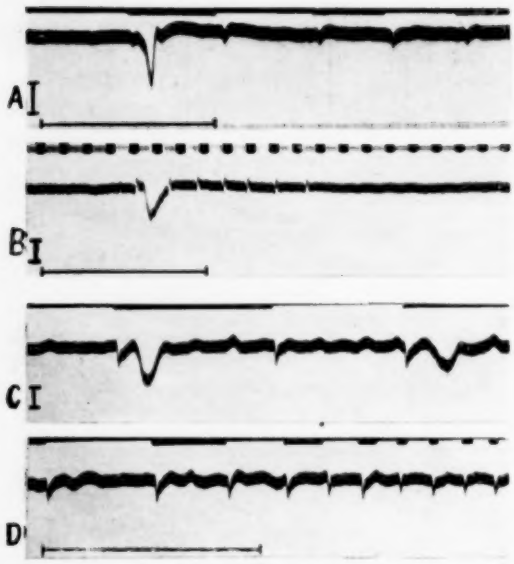

FIG. 4. Effect of repetitive stimulation under deep pentobarbital sodium anesthesia, showing persistence of the primary response and decrease and disappearance of the secondary discharge. A, B, CD, three different experiments. Between C and D, 1 sec. interval. D shows the slight decrease in the primary response on stimulation with higher frequencies. One sec. shown by horizontal lines underneath records. Vertical line beside records represents 100 μV. Time of stimulation (make and break shocks) shown by signal on top of each record.

Another significant fact is that, whereas the secondary discharge varies greatly according to the interval after a previous spontaneous discharge, it appears with similar time relations all over the cerebral cortices. The pri-

mary response, on the other hand, although far less susceptible to fatigue or extinction by previous activity, changes far more than the secondary discharge as the electrodes are moved to different locations. It generally decreases rapidly or completely disappears as soon as the electrodes are more than 6 to 8 mm. away from the point of maximum activity in the sensorimotor area. Figure 5 illustrates this point. This difference is also illustrated in those experiments in which ipsilateral and contralateral responses were

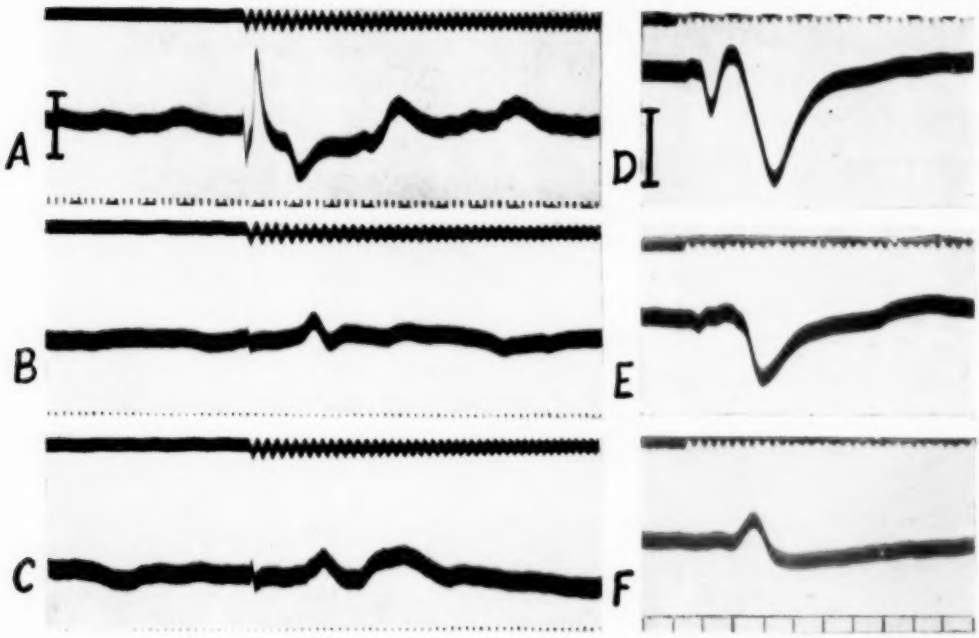

FIG. 5. Responses to sciatic-nerve stimulation from different parts of the cortex under deep pentobarbital sodium anesthesia, showing dependence of primary response on proximity to sensorimotor cortex. A, B, C, one experiment; D, E, F, another. A, D: sensorimotor cortex. B, E: anterior marginal gyrus. C, F: posterior marginal gyrus. *Time* in A, B, C, 0.01 sec., in D, E, F, 0.04 sec., shown below records. Vertical line beside records represents 200 μV. Line on top of records, signal magnet.

compared, either by stimulating alternately right and left sciatic nerves or by recording alternately from corresponding points on right and left hemispheres. They regularly showed greater differences between the primary responses of the two sides than between their secondary discharges. This difference in the majority of our experiments consisted in the complete absence of the primary response on ipsilateral stimulation (Fig. 6). In some experiments this response was discernible but of opposite sign and much smaller than the response to contralateral stimulation. The latency of the ipsilateral response then was usually the same as that of the peak of the primary response on the contralateral side. But following the primary response was a secondary discharge of approximately the same latency and

usually of the same wave-form and magnitude, whichever nerve was stimulated or from whichever cortex it was recorded.

In a few experiments, to a pre-existing deep pentobarbital anesthesia, in which good primary and secondary responses were elicited, ether was added. All these experiments showed no change in the primary response, but a striking increase in the latency of the secondary discharge from about 50 msec. to 150 msec. in about 15 min. The voltage decreased from 150 μV. to 60 μV., after which only the primary response was present. After the ether was taken off, the secondary discharge reappeared with a latency of 150 to 160 msec. and of small voltage. In about 6 to 10 minutes, the voltage and latency returned to their original values (Fig. 7). When ether was added again and continued until death, the primary response persisted long after the second-

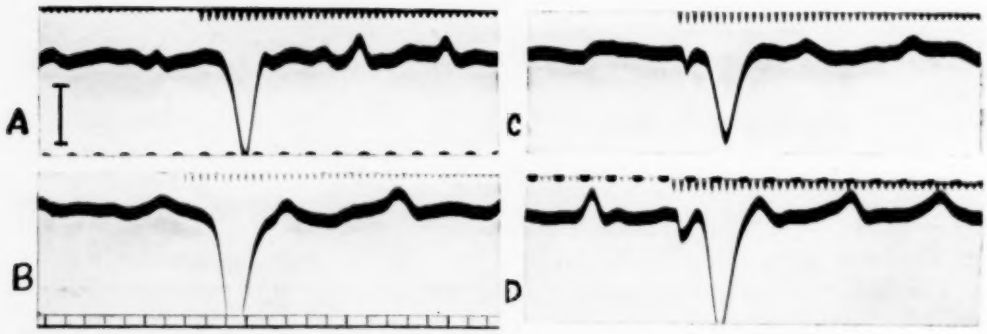

FIG. 6. Differences in responses of sensorimotor cortex to ipsilateral and contralateral stimulation under deep pentobarbital sodium anesthesia. Left column, ipsilateral stimulation; right column, contralateral stimulation. A, C: left cortex; left and right sciatic-nerve stimulation. B, D: left and right cortex; left sciatic-nerve stimulation. *Time* (0.04 sec.) shown below records. Vertical line beside records represents 200 μV. Time of stimulation (single shocks) shown by signal magnet on top of each record.

ary discharge had disappeared; it was abolished just at exitus. Usually great increase in depth of pentobarbital anesthesia caused only a slight increase in latency, although in one exceptional experiment increased pentobarbital narcosis resulted in almost as great an increase of latency as when ether was added (cf. Marshall, 1938).

It has already been emphasized (cf. Derbyshire, Rempel, Forbes and Lambert, 1936, p. 588) that only when the cortex is rendered quiescent by deep barbiturate or similar narcosis do the primary and secondary responses herein described become evident. Some of our experiments show an actual increase in the size of the primary response upon increasing the depth of narcosis, and indeed an even greater relative increase than the corresponding augmentation of the secondary discharge. In one experiment with the needle-plug electrodes, the needle electrode was thrust progressively farther into the gray matter and then through it into the underlying white matter, the ground lead remaining fixed in the cranium throughout the series. The primary response changed little in this series, whereas the secondary discharge

showed a great change in size, form, and phase relations, illustrated in Fig. 8.

From the above observations on latency and localization, it may be inferred that the primary response is associated with afferent impulses in the sensory paths approaching the cortex. The great decline in this response as the electrodes are moved a few millimeters away from the sensory area denotes a fairly sharp localization of this effect in that area. The secondary discharge, on the other hand, appearing about 30 or 40 msec. after the onset of the primary response, occurring in widely separated areas in the cortex, and presenting a similar waveform in corresponding points on the two hemispheres simultaneously, denotes a widespread cortical disturbance which is presumably evoked by the afferent volley. Further evidence concerning the structures involved in these two dissimilar events was sought in a number of extirpation experiments.

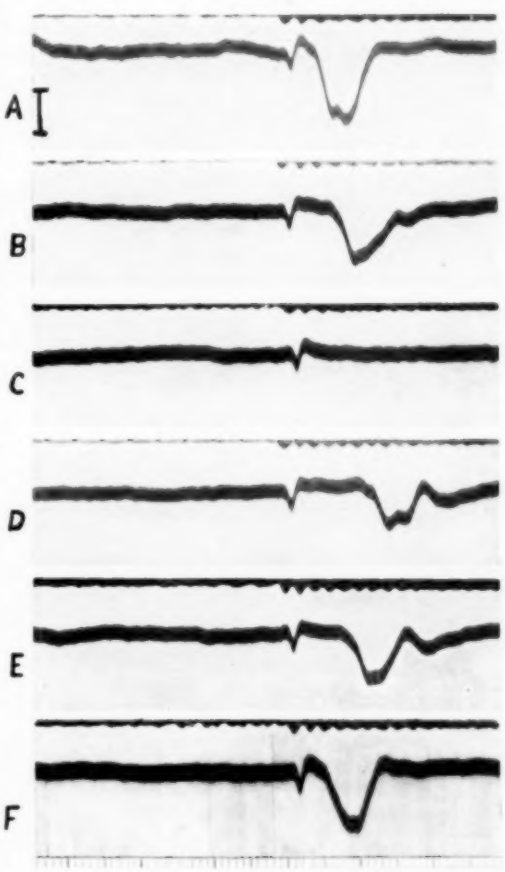

FIG. 7. Effect of ether added to deep pentobarbital sodium anesthesia on the response to sciatic-nerve stimulation. A: deep pentobarbital sodium anesthesia. B: 10 min. after beginning of etherization. C: 8 min. after B. D: 2 min. after withdrawal of ether. E: 1 min. after D. F: 9 min. after E. *Time* (0.01 sec.) shown below records. Vertical line beside records represents 100 μV. Time of stimulation (single shocks) shown by signal on top of each record.

In some experiments we first recorded the response to sciatic-nerve stimulation from the cortex and then removed portions of the cerebrum, exposing the hippocampus or decerebrating the cat. The responses recorded from the afferent path near the anterior end of the hippocampus or from the brain-stem corresponded in their time relations closely with the primary responses from the cortex. This primary response, whether recorded from the cortex or from the afferent path, was sometimes simple, and sometimes dicrotic, as in the corresponding records published by Forbes and Miller (1922). The identification of these two peaks with those appearing in the records of Forbes and Miller is substantiated by the differential effect on them of both ether and repeated stimulation, the second peak always decreasing the more.

The dependence of the secondary discharge on the cerebrum was shown by the following experiment, illustrated in Fig. 9 (ground lead on skull throughout). After recording the responses from both cortices to stimulation of one sciatic nerve, unilateral decerebration was performed, the hemisphere on the same side as the stimulated nerve being removed. The electrode which

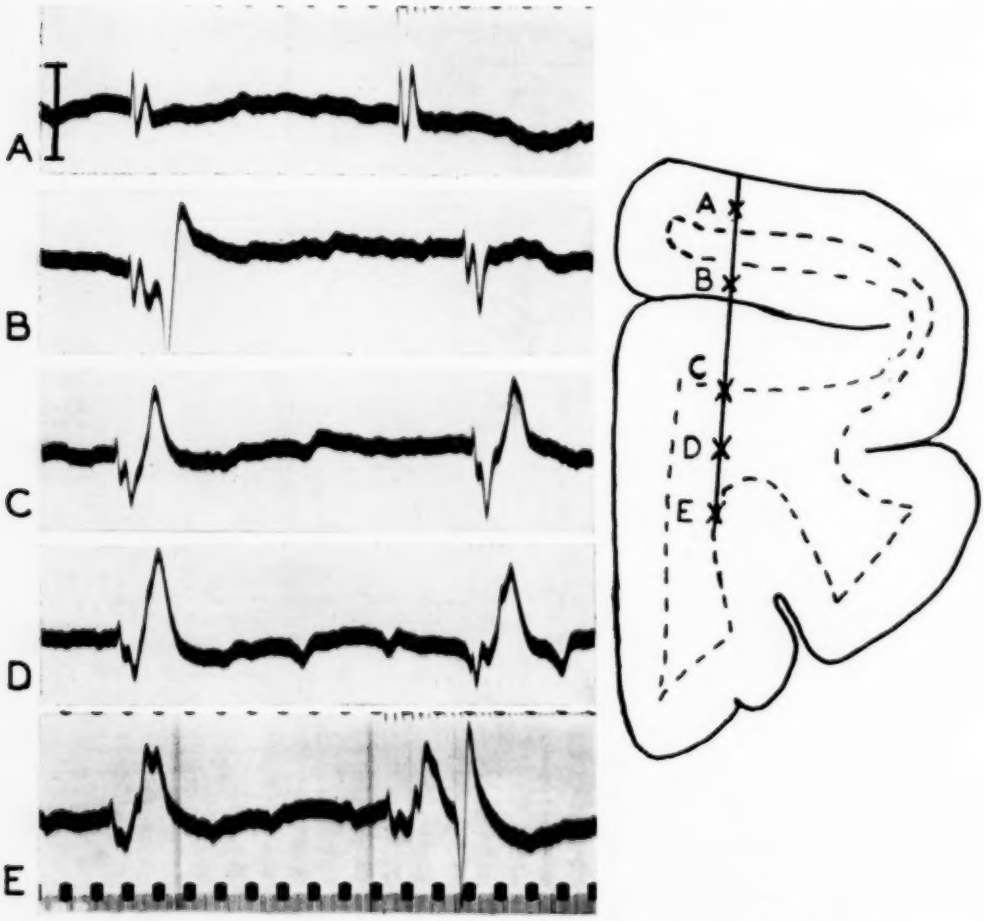

FIG. 8. Changes in the secondary discharge when one electrode penetrates progressively into the brain. Diagram showing section of brain with track made by the electrode. Crosses show approximate position of grid electrode corresponding to each of the records. Ground lead in skull. *Time* (0.01 sec.) shown below records. Vertical line beside records represents 100 μV. In each record are shown the responses to a consecutive pair of shocks, make and break.

had been on that cortex was moved to the cut brain-stem, while the other grid lead remained on the intact cortex. A few minutes after this operation the secondary discharge, led from the intact cortex, showed a latency about three times as long as before decerebration. This latency decreased gradually until at about 10 min. after decerebration it had returned to its original

value. The size and shape then were similar to those recorded before decerebration. When records were taken from the lead on the cut brain-stem the secondary discharge was recorded, greatly reduced in size (Fig. 9B), as would be expected in the case of active tissue remote from the electrode. Decerebration of the second side abolished the secondary discharge on both sides of the brain-stem. A well-defined primary response was then recorded from the side contralateral to the stimulated sciatic nerve. On the ipsilateral

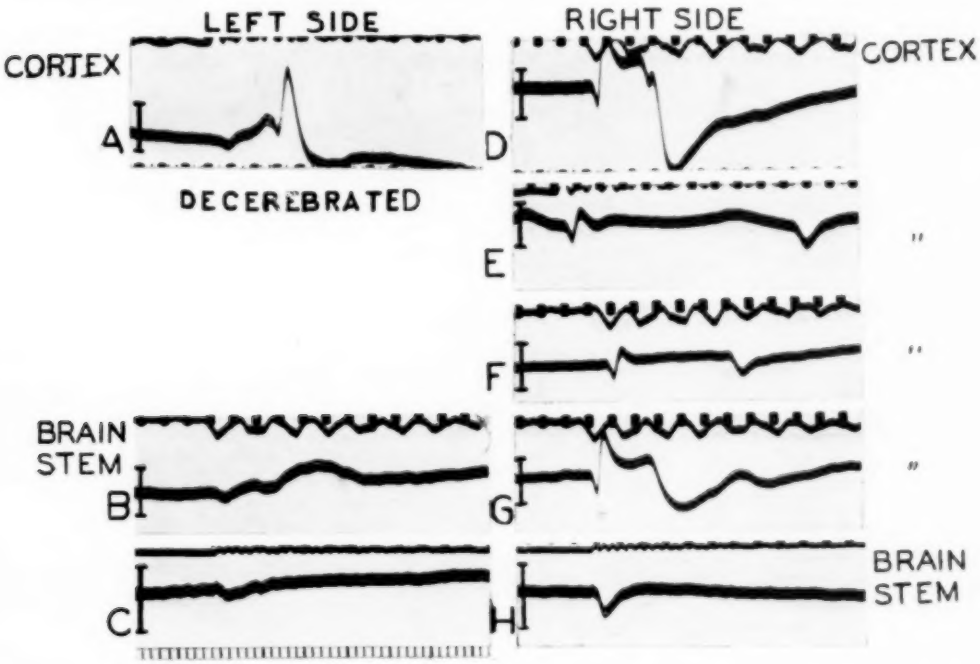

FIG. 9. Effect of unilateral and bilateral decerebration on the responses to sciatic-nerve stimulation. Electrodes: wicks on left and right cortices, grids; plate on skull in midline, ground. A and D: intact cortex; left sciatic nerve stimulated. After A and D, left side decerebrated and electrode from left cortex placed on brain-stem. E, 3 min., F, 4 min., G, 10 min., after decerebration. B record from brain-stem, 14 min. after decerebration. Right side decerebrated. C: left cut surface. H: right cut surface. *Time* (0.01 sec.) shown below records. Vertical line beside records represents 200 μV. Line on top of records, signal.

side only a small primary response was recorded. The large but transient increase in the latency of the secondary discharge after hemidecerebration may have been a consequence of the anemia caused by ligating the carotid arteries and compressing the vertebrals in order to prevent hemorrhage. Beecher, Forbes and McDonough (1938) found that lowering of blood pressure produced changes closely resembling those of increased depth of anesthesia (cf. Adrian and Matthews, 1934, p. 468), and we have mentioned above that these changes sometimes include greatly prolonged latency of the secondary discharge.

In other experiments designed to localize the secondary discharge more

closely in the cortex, several pairs of bipolar electrodes were placed on different parts of the cortex. The usual arrangement was as follows: The first pair of electrodes was placed 2 to 3 mm. posterior to the cruciate sulcus and about 5 mm. from the midline; the second behind the sulcus ansatus on the anterior third of the marginal gyrus; and the third on the marginal gyrus about 10 to 15 mm. posterior to the second. After responses to sciatic-nerve stimulation had been recorded from all three positions, an incision was made

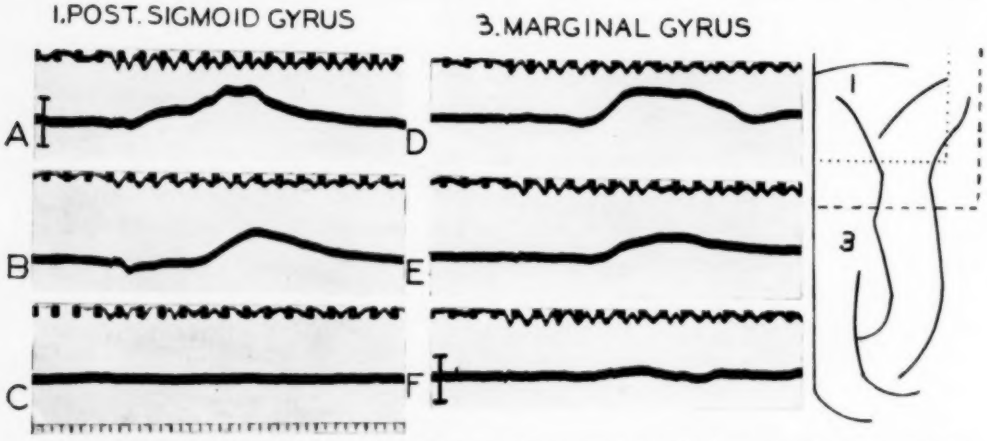

FIG. 10. Effect of cortical extirpations on the secondary discharge under deep pentobarbital anesthesia. Diagram shows positions of the electrodes. A and D, intact cortex. Before B and E, part of cortex anterior to dotted line was extirpated. B, on cut surface beneath position 1. E, still in the same position. Between E and F, corresponding part excised from left cortex. C, after the part of cortex indicated by broken line was extirptaed on both hemispheres. A, B, C, D, E, same amplification. Vertical line beside records represents 200 μV. F, higher amplification. Vertical line in F, 100 μV. *Time* (0.01 sec.) shown below records. Line on top of records, signal.

between electrodes 1 and 2. The responses recorded after that from all three leads were usually decreased in size, but after a lapse of time attained nearly their previous size. A second incision between electrodes 2 and 3 had the same effect. Removal of the cortex anterior to the first incision decreased the responses from the cut surface to which electrode (or electrodes) 1 was reapplied, and even more so from the posterior electrodes which were still in place. Small secondary discharges on the operated side, and larger ones on the intact side, continued to be recorded until the frontal part of the cortex as far as the anterior third (approximately) of the marginal gyrus on both sides had been removed. With this extirpation the secondary discharge was completely abolished. Such an experiment is illustrated in Fig. 10.

DISCUSSION

The experiments of Marshall, Woolsey and Bard (1937; see also Bard, 1938) with sharply localized tactile stimuli, in both cat and monkey, show correspondingly localized cortical potentials. Their latencies and durations are nearly the same as those of the primary response in our experiments. On repeated stimulation the responses decrease in size and disappear at fre-

quencies of from 12 to 15 per sec. We have not used such high frequencies as this, but, since with frequencies up to 7 per sec. the primary response continues to appear with more than half its initial size, it seems unlikely that at double that frequency our primary responses would decline to extinction. Probably the conditions of our experiment—*viz.*, strong stimulation of the entire sciatic nerve—are more favorable to persistent response. Possibly the response which Bard and his co-workers have recorded is from a mechanism intermediate between those whose activity is patent in the primary response and secondary discharge in our records, and is more localized than our widespread secondary discharge.

The time of the primary response has been mentioned loosely as showing the time of "arrival in the cerebrum" of the afferent volley. It is desirable to be more specific, if possible. Leese and Einarson (1934) showed that the first response in the medulla oblongata, when the sciatic nerve was stimulated as in these experiments, appeared about 6 msec. after the stimulus. Forbes and Miller (1922), recording from the cut brain-stem at the colliculi, found after the medulla response a second excursion whose peak was attained at about 20 msec. after the stimulus. We find that whether we lead from the sensorimotor cortex or from the afferent path near the anterior end of the hippocampus, the first excursion begins about 10 msec. and reaches its peak about 20 to 22 msec. after the stimulus.* In those preparations which we decerebrated after recording from the cortex, the major excursion recorded from the brain-stem showed approximately these same time relations. The conclusion seems to be that conduction from the level of the colliculi to the sensory cortex is rapid. The peak of the wave recorded from the sensory area probably represents the actual arrival of the afferent impulses, and we may infer that they reach the cortex not over 25 msec. after the stimulus is applied to the sciatic nerve. Moderate slowing of the primary conduction time has sometimes been found under deep barbiturate narcosis. Much more marked is the slowing of the secondary discharge under like conditions. In general the latency of the latter is much more variable than that of the primary response. The extreme example of this is the increase of latency to 150 msec. when ether is superimposed on barbiturate narcosis.

Probably the most significant feature of the secondary discharge is its complete disappearance when the stimuli are applied with frequencies greater than 3 or 4 per sec. This effect suggests a compound rather than a simple system. If a nerve or muscle is stimulated with a frequency higher than it can follow, it will respond rhythmically at a frequency determined by its refractory period. This is most strikingly shown by the rhythmic contractions of the heart in Stannius ligature when a rapid succession of stimuli is applied. The behavior of the secondary discharge in the cortex is entirely different. A rapid series of stimuli evokes only one response; as long as the stimuli continue there is no recurrence.

* In the most clearly defined records of this type the localizing electrode in the sensory path became negative.

Perhaps the simplest way to explain the disappearance of the secondary discharge on rapidly repeated stimuli would be to suppose that it is evoked through some mechanism whose threshold is such that only after a rest is the afferent volley (primary response) strong enough to be an adequate stimulus, and that with the demonstrable decline in the primary response to a depressed level it falls below the threshold. To this explanation, it might be objected that the afferent volley when it reaches the cortex consists of impulses in axons responding on the all-or-none principle, whose relative refractory periods are much less than 0.25 sec. This may be answered by the probability that the *number* of converging impulses determines the strength of the volley as a stimulus to the cortical synapses. Indeed the obvious fact that the primary response declines when repeated more than 5 times a second shows that the available energy is in some way reduced. A more serious objection to the simple explanation proposed is that the observed decline in the primary response is relatively small—often not more than 20 or 25 per cent. With so small a difference in strength between the first and subsequent volleys, one would expect the extinction of the secondary discharge after all but the first volley of a series to occur only in a narrow range of depths of narcosis, for soon after the excitability had decreased enough to render the later volleys ineffective its further decrease would render even the first (full-sized) volley ineffective. But this is not the case. The secondary discharge is found over a wide range of depths of narcosis, and as far as our observations have gone they seem to show that whenever it can be evoked it can also be made to disappear on repetition of stimuli.

As intimated in a previous paper, the effect may perhaps be the result of inhibition. If so, the inhibitory effects of the afferent volleys must continue to dominate the discharge as long as these are delivered above the requisite frequency of 4 or 5 per sec.

The great mass of data which we have gathered concerning the "alpha rhythm" of Berger suggests the existence of a "pace-making" mechanism which tends to synchronize the discharge of great quantities of cortical cells. The evidence of Derbyshire, Rempel, Forbes, and Lambert (1936) that pentobarbital in its early stages increases the amplitude of the major spontaneous waves without disturbing their rhythm suggests that the tendency of large groups of cells to synchronize is increased by moderate doses. As the narcosis deepens, the waves become more infrequent but show little change in their individual time relations. Forbes, Renshaw and Rempel (1937) described deep stages of pentobarbital narcosis in which isolated waves, individually of similar time relations to those in the lighter stages, rise from a smooth base line, sometimes at regular intervals, but in the deeper stages at long and irregular intervals. In the deepest stages these spontaneous waves disappear and the base line is continuously smooth.

The secondary discharge can be evoked at any stage of narcosis which offers a smooth base line even for a fraction of a second, but, as already noted, the spontaneous waves prevent the discharge from appearing if the

stimulus is applied too soon after their cessation. This strongly suggests that the same pace-making mechanism which synchronizes the spontaneous discharge of the cells may also initiate the widespread disturbance which constitutes the secondary discharge. This is further suggested by the fact that the spontaneous waves, though sometimes dissimilar, often closely resemble the secondary discharge in direction, duration and wave-form. The pace-making mechanism might be pictured as normally active and initiating waves of more or less regular rhythm, and becoming progressively less prone to discharge as narcosis deepens, although the cells they synchronize are still able to respond. The pace-maker would then discharge less frequently until finally it became quiescent. But even then it could be stimulated by an afferent volley and thus made to set off the secondary discharge. If we further suppose that the pace-maker is subject to rapid fatigue, we can explain its failure to set up more than one secondary discharge when afferent volleys arrive at excessive frequencies. But it will not suffice to ascribe the effect simply to a long refractory period in the pace-maker, for in that case it would recover and initiate discharges later in the series of stimuli. Two explanations of its continued failure throughout the series occur to us. We may suppose either that the refractory phase of the pace-maker, set up by the first volley, is maintained by some effect analogous to the post-cathodal depression described by Erlanger and Blair (1931), or that the pace-maker is excited by each volley to some degree of activity, but that narcosis and fatigue conspire to make its response too small to initiate the widespread discharge.

Bishop and O'Leary (1936) showed that in the lightly etherized rabbit, stimuli applied to the optic nerve evoke in the optic cortex surface-positive waves of somewhat similar time relations to the secondary discharge which we have observed. They ascribe them to corticifugal impulses travelling to the thalamus. More recently three sequences of potentials have been identified in the optic cortex (Bartley, O'Leary and Bishop, 1937, rabbit; Bishop and O'Leary, 1938, cat). Application of strychnine has furnished reasons for associating these sequences with different neural mechanisms. In both animals the slowest waves (ascribed to corticifugal impulses) are taken to involve the same mechanism as the alpha waves. In our experiments the long duration of the secondary discharge, its wide distribution and its apparent involvement of the same mechanism as spontaneous discharges resembling alpha waves, all suggest that it is of the same kind as the slowest waves described by Bishop and his co-workers. Like these it may well be a corticifugal discharge, and in favor of this view is the fact that when recorded with a large surface electrode referred to a ground lead on remote inactive tissue, the secondary discharge is regularly surface positive in all regions of the cortex close to the sensorimotor area.

Adrian (1936), stimulating the rabbit's cortex directly with brief electric currents, has reported a spread of surface-positive waves, referable to activity of cells in the deeper layers of the cortex, radiating from the stimulated point. If we look on the secondary discharge as a similar spread from the

sensory area, we encounter two difficulties. The discharge does not appear surface-positive in all parts of the cortex, but presents differences in shape and sometimes in polarity in different areas (cf. Derbyshire, Rempel, Forbes and Lambert, 1936). More difficult is the fact that, although there is a lag of 20 to 50 msec. between the primary response and the secondary discharge at the sensory area, the secondary latency usually increases so little as the leads are placed farther away from the sensory area as to indicate conduction velocities of 50 to 100 cm. per sec., which exceed the range of velocities (10 to 40 cm.) observed by Adrian. These facts suggest that the disturbance spreads over the cortex via some more rapidly conducting channels than those chains of neurons which were involved in propagating the waves described by Adrian, or that the velocities in corresponding chains are more rapid in the cat than in the rabbit. A third alternative is that the afferent paths deliver the incoming volley not only to the sensory cortex, but also the thalamus or other subcortical centers, and that from there impulses are distributed widely in the cerebral cortex. This explanation would readily account for the delay of 20 msec. or more after the arrival of the afferent volley before the appearance of the secondary discharge in the sensory cortex. It would also explain the brief and variable interval between the arrival of the secondary discharge at the sensory area and at more remote regions of the cortex, for the subcortical centers can easily be supposed to deliver this disturbance in many parts of the cortex almost simultaneously. Incisions in the cortex between the sensory and other areas have furnished evidence both for and against this view. In some cases they have resulted in a great decrease in the discharge recorded from the regions thus cut away from the sensory area. In other cases the discharge has persisted with little change after such incisions have been made. There is need for further research on this particular problem.

Bremer (1937) has argued that barbiturates act selectively on internuncial neurons in the cortex, rather than on afferent or motor paths. The secondary discharge, representing cortical activity which finds no motor expression in the skeletal musculature, would seem to be most easily explained as a discharge of internuncial neurons, and is therefore hard to reconcile with Bremer's view. But the structure of the cerebrum is so complex and includes so many different kinds of neuron that one can conceive of one type being narcotized while another type remains excitable. It is not certain, therefore, that our evidence cannot be reconciled with Bremer's view.

Perhaps the quiescent state of the cortex under deep barbiturate narcosis, even when it is clearly capable of activity in response to sciatic stimulation, may be explained by a rise in threshold in its channels of approach such that only massive stimulation can break through. Possibly, as Beecher, Forbes and McDonough (1938) have suggested, the ordinary streams of sensory impulses fail to excite the cortex because of its high threshold, yet the mass of convergent impulses due to maximal stimulation of the sciatic nerve produce a central effect adequate to attain the threshold and evoke a generalized discharge.

Summary

1. A previous paper described a widespread electric response of the cerebral cortex to stimulation of the sciatic nerve appearing in the deeper stages of narcosis with pentobarbital or avertin. We propose to call it the "secondary discharge." The same effect is found with dial (di-allyl barbituric acid).

2. The secondary discharge is invariably characterized by its immediate and complete extinction when the initial stimulus which evokes it is followed by succeeding stimuli at frequencies above about 3 per second, by the need of a rest of considerably more than a second before a full-sized discharge can again be evoked, by its subnormal magnitude if evoked at intervals between about 0.5 sec. and at least 1.5 sec., and by its complete failure to reappear after its initial extinction even if the rapid series of stimuli is continued for several seconds. In like manner, shortly after a spontaneous wave the secondary discharge cannot be evoked. In these respects it is sharply differentiated from the primary response which precedes it, whose magnitude declines only slightly in the successive responses to rapidly repeated stimuli.

3. The secondary discharge is widespread in the cerebral cortex, appearing with nearly the same latency and duration in both hemispheres in response to stimulation of one sciatic nerve, and with similar time relations (though sometimes with reversed polarity) in regions of the cortex remote from the sensory area.

4. On stimulation of one sciatic nerve, the primary response differs far more when compared in the two hemispheres than does the secondary discharge which follows it. A few millimeters away from the sensory area, the primary response may not be discernible, although in the same preparation it exceeds the secondary discharge in voltage when recorded from the sensory area for the sciatic nerve (Fig. 5).

5. The latency of the primary response in the sensory cortex identifies it with the afferent volley, which can be recorded at various points in the afferent path from the medulla to the cerebral cortex.

6. Incisions through the cortical gray matter between the sensory area and other regions to which electrodes were applied, in some cases reduced the response in a manner that suggested a spread of the disturbance from the sensory area by way of the cortical gray matter. But the long delay between the primary response and the secondary discharge, and the approximate synchronism of appearance of the discharge in widely separated regions of the cortex, suggest that the afferent volley may act upon the thalamus or other subcortical centers, and that these centers may then distribute the discharge throughout the cortex.

7. The failure of all but the first of a series of repeated stimuli above a critical frequency to evoke the discharge suggests the existence of an intermediate mechanism between the afferent tract and the cortical cells whose discharge is recorded. A pace-making mechanism, normally producing the synchronized rhythmic discharge of cortical cells, having its threshold raised by the narcotic and in this state rapidly fatigued, might be the intermediate

mechanism whose failure would explain the extinction of the response to repeated stimuli.

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A SEARCH FOR CHANGES IN DIRECT-CURRENT POTENTIALS OF THE HEAD DURING SLEEP

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INTRODUCTION

DURING SLEEP the pattern of electrical activity recorded from the human head differs widely from the waking pattern (Loomis, Harvey, and Hobart, 1937, 1938). As sleep becomes deeper the electrical waves increase in voltage and become longer and longer until the time from crest to crest becomes as great as 4, 6, and even 10 sec. Such slow changes of potential tax the ability of available capacity-coupled amplifiers (time constant = 0.5 sec.) and suggest the possible existence of still slower changes, perhaps even changes in steady state directly related to fluctuations in the depth of sleep. Slow changes in the potentials of the brain (so-called "D.C. changes") correlated with changes in physiological state are also suggested by the observations of Cohn and Langenstrass (1938) on patients in insulin coma and by Burge, Wickwire, *et al.* (1936) on animals under various depths of anesthesia. Another instance of systematic change in D.C. potential is the "ovulation potential" (Burr, Hill, and Allen, 1935; Reboul, Davis, and Friedgood, 1937). The transient "galvanic skin reflex" is a familiar phenomenon. Because of these various suggestions of the existence of significant D.C. changes correlated with changes in physiological state we undertook to observe the changes in D.C. potentials on the human head during sleep.

METHOD

Apparatus. Two fundamentally different methods of amplifying and measuring D.C. potentials have been employed. The first is a push-pull direct-current amplifier and mirror galvanometer. The scale is conveniently read to 0.5 mm., corresponding to 0.05 mV. of potential-difference between the input leads. The amplifier is stable and convenient in operation. The input is balanced and a large cathode resistor is used (Fig. 1); consequently the instrument is quite insensitive to movements of the subject and to all unwanted (in phase) potentials. It is unnecessary to ground the subject or to take any special precautions to insulate him from ground. Stability of the amplifier is improved by never turning the set off, as the cooling and reheating of the tubes is liable to change the balance of the set. A trickle charger keeps the filament battery charged.

The other method depends upon converting a steady potential-difference into a pulsating current by means of a mechanical interrupter. The brief pulses are accurately amplified by condenser-coupled amplifiers and recorded simultaneously with the usual electroencephalogram by means of a multi-channel ink-writer (Loomis, Harvey, and Hobart, 1938). To use this latter method on our regular 6-channel oscillograph it was only necessary to connect a motor-driven short-circuiting switch across the input of each circuit desired to use as a D.C. circuit. The input of each amplifier was thus short-circuited 5 times a sec. When the short-circuiting switch was closed, there was no voltage between the grids of the first pair of tubes. When the short-circuiting switch opened, the D.C. potential which we wished to measure appeared between the input leads, and the recording pen made a corresponding excursion. The pen returned to its base-line when the switch closed again.

Carefully prepared silver contacts proved adequate to avoid trouble from contact potentials when the short circuit was closed. The sensitivity of the amplifiers was adjusted so that when there was 1 mV. across the input the amplitude of the square waves produced by the opening and closing of the short-circuiting switch was 1 cm. Thus all D.C. voltages of less than 1 mV. could be read directly from the record. For larger voltages a compensating voltage to the nearest even millivolt was placed in series in the input circuit and the number of millivolts thus added was automatically recorded on the record. The great advantage of this method for long runs is that the calibration is dependent only on the compensating voltage and is not dependent in any way on the "drifting" of the amplifiers.

Electrodes. Electrodes present a special problem in studies of D.C. potentials. In preliminary experiments silver-silver chloride half-cells were employed resembling those de-

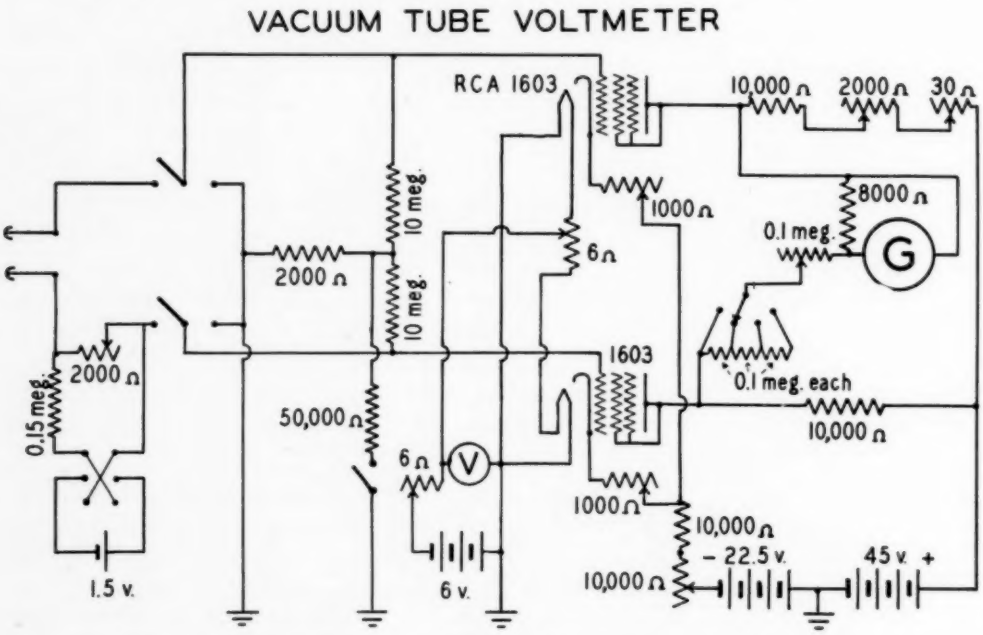

FIG. 1. Wiring diagram of balanced-input D.C. millivoltmeter.

scribed by Burr, Lane, and Nims (1936). Such electrodes are stable and equipotential far beyond the requirements of the present type of experiment, but are difficult to attach to the subject without leakage from the salt bridges. They are excellent for brief explorations, but troublesome for a night's sleep. Miniature Ag–AgCl–NaCl electrodes were devised, each one less than 1 cm. in height and diameter. These were small cup-shaped cells which were molded from transparent methyl methacrylate in such a way that there was a long spiral capillary tube in the wall of the cell, opening to the exterior. The capillary allowed for contraction and expansion of the fluid within, without escape of fluid or admission of air. These cells were satisfactory in their electrical stability and for short runs, but it was difficult to keep them on a subject's head when he tossed during sleep. Two fairly simple and mechanically convenient combinations with adequate electrical stability were evolved, one employing zinc, the other copper, as the metal.

From sheet copper, 0.125 mm. thick, spoon-shaped pieces about 20 mm. long are cut. The "handles" are 1.5 mm. wide, the bowls are circular, 6 mm. in diameter. Enamel-insulated copper wire (No. 32) is attached to the handle by bending the sheet copper over the bared end of the wire and squeezing. This joint involves no other metal than copper, but it is desirable to wrap joint and handle with "scotch tape" (cellophane and an adhesive) to restrict the area of electrical contact to the disc. The copper is cleaned with acid, and just before application is cleaned again with emery paper. The electrolyte is copper

sulphate (4 per cent) in agar (1.5 per cent) jelly. Two drops of glycerine per cc. are added to the jelly to delay drying. Bits of filter paper slightly larger than the discs are impregnated with the jelly and laid on the skin, which has previously been cleaned with acetone or ether. The electrode is attached to the skin by a layer of collodion which completely covers the electrode, both disc and handle. The mechanical attachment is satisfactory, particularly on the scalp where a few hairs can be drawn across the copper strip for reenforcement. It is not necessary to cut any hairs if jelly is applied liberally beneath the filter paper. The metal strip can easily be bent to conform to the shape of the head. Such copper strips immersed in the $CuSO_4$-agar jelly in a petri dish rarely show potential-differences of as much as 1 mV. after the first minute. The differences which do appear are usually stable within 0.2 mV. over several hours. They show only very slight back E.M.F. after the passage of a D.C. current of 0.5 mA. The chief disadvantage of this type of electrode has been slow drying of the jelly.

The zinc electrodes are similar in size and general design to the copper electrodes already described. The zinc is lightly amalgamated by dipping in a dilute $HgCl_2 - HCl$ mixture for a few seconds. The solder junction to the copper wire must be carefully protected by insulating lacquer and scotch tape. The "bowl" of the spoon is hollowed slightly to aid in retaining the electrolyte paste. A fairly satisfactory paste is Sanborn "Redux" electrode paste to which has been added 1 gm. of $ZnSO_4$ per 25 gm. of paste. This paste retains its moisture far better than agar jelly, and is more convenient to keep and to apply. It cannot be used with copper, however, as it reacts with the cupric ion. One disadvantage of zinc is that the zinc electrodes are not perfectly reversible. Current-flow is not proportional to applied voltage for currents of the order of 0.1 mA. at 0.01 volts, and there is a significant back E.M.F. following the passage of such a current. The potential-differences among a number of strips dipped in paste may be as much as 3 mV. and are a little less stable than with copper. The differences and the variability are small, however, compared with the potential-differences and fluctuations introduced by the human skin, and in spite of their obvious imperfections we have employed the $Zn–ZnSO_4$-paste electrodes in nearly all of the experiments to be described below.

In 7 experiments electrodes were placed on the head in the following positions: left and right frontal, 6 cm. from the midline at the level of the usual hair-line; left and right central, 6 cm. from the midline in the frontal plane of the auditory meatus; right and left occipital, 5 cm. from the midline at the level 2 cm. above the inion; right and left "ears" just behind the ear and above the mastoid process. One or two electrodes were also placed on the upper chest near the midline at the upper end of the sternum.

One electrode, usually on the chest, was chosen for reference, and the potential-differences between this and each of the other electrodes were measured periodically, usually every 15 min., by means of the vacuum-tube millivoltmeter. On the 6-channel ink-writing oscillograph, three or four channels were used to record the usual A.C. brain-waves from various parts of the head. The remaining three or two channels were used with motor-driven short-circuiting switches to record the D.C. potentials from three or two other regions.

Results

The electrodes when first placed on the head and chest always showed potential-differences far greater than those found in control comparisons made in electrolyte paste immediately before applying them. Most of the potential-differences after application were less than 5 mV., but some were as much as 20 mV. The deviations showed no relation to positions of the electrodes or to the electrical resistances (15,000 to 30,000 ohms) between them. We attribute the deviations to local accidental differences in the condition of the skin beneath the electrodes, although the skin was cleaned thoroughly but gently and care was taken to avoid all cuts or abrasions. The potential-differences often shifted gradually by 3 or 4 mV. during the first half-hour, whether the subject remained awake or went to sleep, and then either remained quite stable (within less than a millivolt) or attained a slow

rate of drift which might be maintained steadily for hours (Fig. 2 and 3). Neither the initial shifts nor the more prolonged drifts could be related to the position of the electrode or to any other factor which we have been able to identify.

Relation of D.C. potentials to movement. Movements of the subject sometimes caused changes in the D.C. potentials. Often there was a transient shift of 2 or 3 mV. Sometimes the return was slower, requiring from 2 minutes to half an hour (Fig. 3C). All of the abrupt changes in D.C. potential which were observed were associated with movements. There were, however, numerous minor slower fluctuations, of as much as 1 mV. over periods of 1 to 5 min., superimposed on the slower drifts, which could not be related to any apparent cause.

Relation of D.C. potentials to sleep. When the subject fell asleep, as he passed from light to deep sleep, or when he awoke, either gradually or abruptly, no change whatever could be observed in the D.C. potentials of the head. There was no systematic change either between head and chest, between scalp and ears, between frontal and occipital regions or between right and left. Movements occasionally caused their typical abrupt shifts or momentarily obscured the record, particularly on sudden awakening,—but, sleeping or waking, the D.C. potentials between any of the pairs of electrodes continued their slow random drifts. The negative result is consistent in all 7 experiments. We find no change in the D.C. potential-differences or in their "spontaneous" variations which can be related to the state of sleep. In spite of the drifts and the more abrupt alterations resulting from movement, our conclusion can be drawn with confidence for two reasons. First, the large number of electrodes applied to each subject allows us to identify and discount the occasional erratic behavior of an individual electrode and also to average the random drifts of all of them. Occasional D.C. changes at one or two electrodes which occur coincidentally with waking or going to sleep are not reflected at corresponding electrodes on the opposite side of the head and are not reproduced in other experiments. Second, the major changes in potential are either much slower than the changes in the state of sleep, or else (when associated with movement) they are much quicker.

The usual electroencephalogram reveals characteristic alterations in sleep (Loomis, Harvey, and Hobart, 1937; Blake and Gerard, 1937), so that the depth of sleep can be estimated from minute to minute with considerable accuracy. Figures 2 and 3 show graphically the progress of D.C. potential-differences in 3 experiments and the lack of any relationship to the character of the pattern in the electroencephalogram. In all 3 experiments the subject passed from the waking state (*A*) to the deepest (*E*) stage of sleep within a period of an hour and a half or less.

Figure 2 shows the potentials from the entire series of electrodes, referred to the electrode on the left mastoid as reference electrode, determined periodically by the voltmeter in an all-night experiment. The subject was unusually tired. She had had a total of only 22 hours sleep during the 4

previous nights and had driven an automobile 225 miles between noon and 7:30 p.m. on the day of the experiment. She reported sleeping soundly all night and was still asleep when the readings were taken at 8:15 a.m. next morning. After breakfast she returned to the experimental room for a second nap. The electrodes remained undisturbed throughout.

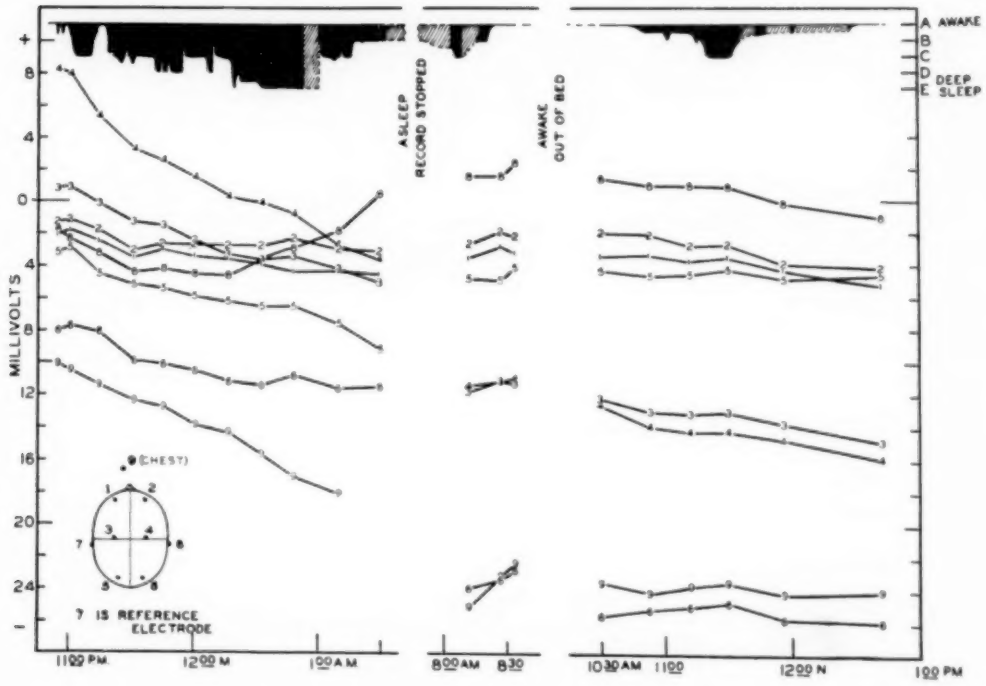

FIG. 2. Differences of potential in millivolts measured by balanced D.C. millivoltmeter between electrodes on head and chest of a 40-year-old woman during the first part of a night's sleep and during a nap the next morning. Positions of electrodes are indicated by diagram in lower corner. Zn–$ZnSO_4$-paste electrodes. In this and subsequent figures the depth of sleep is evaluated according to the criteria of Loomis, Harvey, and Hobart (1937) from a simultaneous electroencephalogram. The record was stopped during the intervals indicated by the gaps and by the shaded areas in the sleep chart.

Figure 3A is constructed from the continuous D.C. record of the potential-difference between left occiput and left mastoid taken during the first part of this same experiment. The more rapid initial changes and the steady subsequent drift with minor fluctuations superimposed on it are well illustrated.

Figure 3B is a similar record of the potential-difference between chest and left central area in another subject during the first part of a night's sleep. This subject was also fatigued from driving an automobile continuously for 11 out of the previous 12.5 hours. The progressive negative trend of the scalp which occurred in this case did not appear in other experiments.

Figure 3C shows the course of the potential-differences between right

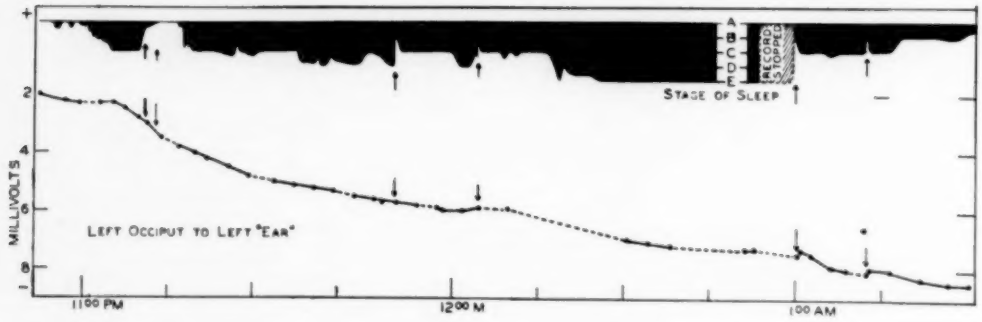

FIG. 3A

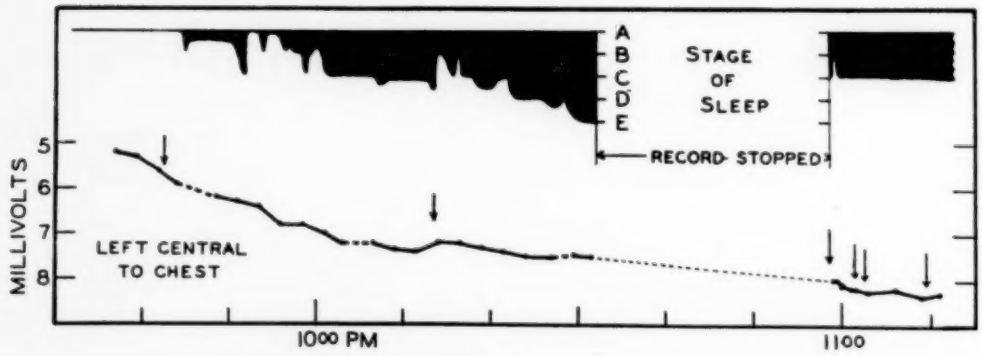

FIG. 3B

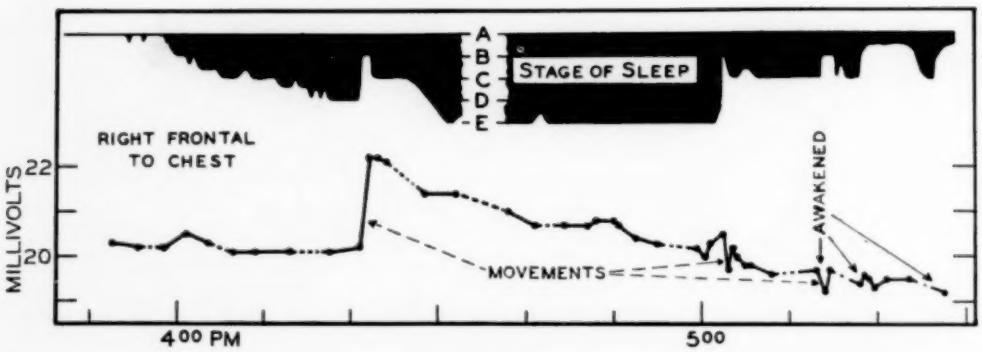

FIG. 3C

FIG. 3. Potential-differences recorded during sleep between an electrode on the scalp and a reference electrode on chest or mastoid. Plotted from continuous records obtained by means of interrupter, capacity-coupled amplifier, and ink-writer. Zn–$ZnSO_4$-paste electrodes. Where solid lines are drawn in the graphs the records of potential were continuous and showed no deviations of more than 0.1 mV. from the lines drawn. The solid circles represent readings from the tape at arbitrary intervals and at extremes of fluctuations. The continuous D.C. record was stopped during the intervals represented by the dotted lines to allow measurements by the D.C. millivoltmeter.

A: 40-year-old woman; same experiment as Fig. 2. Arrows indicate movements of the sleeper.

B: 37-year-old man; first part of night's sleep.

C: 21-year-old man; afternoon nap.

frontal regions and chest in another subject during an afternoon nap. It illustrates the sudden shift produced by a gross movement and the slow drift back to the original level.

The results of the present investigation fail to disclose any change in D.C. potentials of the head associated with the state of sleep. We plan to explore other parts of the body, especially the palms of the hands and soles of the feet (Forbes and Andrews, 1937), to see whether electrical changes in these other regions can be correlated with states of sleep.

Summary and Conclusions

Two methods of measuring D.C. potentials from the human subject are described, one a push-pull vacuum-tube millivoltmeter, the other an adaptation of the usual type of capacity-coupled ink-writing oscillograph. The second method is based upon mechanical interruption of the input potential. The sensitivity of both methods is about 0.05 mV.

Convenient electrodes of the $Zn\text{-}ZnSO_4$ and also $Cu\text{-}CuSO_4$ types are described. Neither combination is entirely satisfactory, but the errors introduced are small compared with potential-differences arising apparently in the skin.

No correlation could be detected between the stage of sleep and the D.C. potential-differences or changes in D.C. potential observed between chest and head, scalp and mastoid region, frontal and occipital regions, or right and left sides of the head.

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ON THE INFLUENCE OF ANOXIA ON PUPILLARY REFLEXES IN THE RABBIT*

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ALTHOUGH EXTENSIVE STUDIES have been published by Gellhorn and collaborators (1934–1937) on the effect of anoxia on cortical phenomena, subcortical reflexes have been but little investigated. In 1935 Gellhorn and Spiesman showed, in experiments on the effect of caloric stimulation of the vestibular apparatus, that under the influence of low oxygen tension nystagmus was decreased. The effects were reversible. It was noted, however, that much lower concentrations of oxygen were needed to bring about these effects than were required for influencing cortical activities. In a recent study Gellhorn and Storm (1938) investigated the influence of anoxia and related conditions on the galvanic nystagmus in the rabbit. They found, in narcotized animals that anoxia predominantly decreases the galvanic response, whereas in the non-narcotized animal an increase of the galvanic response prevails. The results suggest that the effects of oxygen lack on subcortical processes are modified by the cerebral cortex.

It seemed desirable, therefore, to reinvestigate the problem of the effects of anoxia on subcortical structures. In view of the fact that Karplus and Kreidl (1911) assume in the rabbit that pupillary dilatation following pain stimuli is due to an inhibition of the parasympathetic (and not to excitation of the sympathetic), the rabbit was chosen for study. This makes it possible to extend our investigations to other subcortical reflexes and also to include central inhibitory processes in the investigations. Our earlier experiments indicated that relatively small concentrations of CO_2 offset the effects of anoxia (Gellhorn, 1936–37). This was true, not only for cortical but also for subcortical processes (Gellhorn and Storm, 1938). The mechanism involved is largely the synergistic effect exerted by low oxygen and CO_2 tension on the vasomotor apparatus (Gellhorn and Lambert, 1939). Similar experiments on the pupillary reflex dilatation in the rabbit are now reported.

METHOD

Two groups of rabbits were studied: (i) five normal animals; (ii) six animals with unilateral cervical sympathectomy, and one with bilateral sympathectomy. The operation was performed by excision of approximately 1 cm. of the cervical sympathetic in the neck. All animals had a persistent smaller pupil on the operated side under conditions of equal illumination. For the experiments the animals were injected with 0.8 gm. urethane per kg. subcutaneously, and the sciatic nerve was exposed and shielded electrode applied. The preparation was then strapped on to a rabbit board, and the head placed in a modified head holder which permitted the breathing of a gas mixture from a Douglas bag. The

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experiment was begun several hours after the injection of the anesthetic and was repeated several times in each preparation.

Stimuli were delivered by a Harvard inductorium and one dry cell, the stimulus lasting 3 sec. Under these conditions the threshold for the pupillary dilatation lay usually between 11 and 12 cm. on the induction coil. Occasionally the coil had to be rotated to give a threshold stimulation. The eyes were held open by tape, and for 0.5 min. preceding the stimulation a strong light was flashed into the eye. The diameter of the pupil was measured before and after stimulation by a small microscope fitted with a graduated scale, on which one division represented 0.5 mm. in pupillary diameter. A pupillary dilatation of 2 divisions was considered threshold, as the stimulus necessary to produce this reaction could be sharply delimited. Pupillary reactions below this magnitude gave inconstant values. In all experiments the pupillary dilatation was the first sign of the animals' response to the stimulation. If stimulation at 9.0 cm. gave pupillary dilatation, 8.5 cm. gave muscular twitchings, and 8.0 cm. vocalization. The experiments on the *unilaterally* sympathectomized animals were slightly modified. Both eyes were observed simultaneously by two observers and the stimulation time reduced to one second. Hereby the determination of the threshold became more precise. In order to avoid tilting the induction coil, a resistance was introduced into the stimulating circuit. This gave a threshold reaction between 8 and 10 cm. coil distance.

Results

The experiments on the influence of anoxia on the pupillary reflex dilatation in normal rabbits are summarized in Table 1. It is seen from this

Table 1. Normal animals.

No. of expt.	Air control threshold	Threshold during O_2-lack	O_2 conc. per cent	Air control threshold	Threshold during O_2-lack plus 4 per cent CO_2	Air control threshold
1.	8.0 cm.*	7.0 cm.	8	8.0 cm.	8.0 cm.	8.0 cm.
2.	13.0 cm. and 45°	12.5 cm.	8	13.0 cm. and 45°	13.0 cm. and 45°	13.0 cm. and 45°
3.	12.0 cm.	10.0 cm.	7	12.5 cm.	11.5 cm.	12.5 cm.
4.	12.0 cm.	10.5 cm.	7	12.5 cm.	12.5 cm.	12.5 cm.
5.	11.5 cm.	10.0 cm.	7	11.5 cm.	11.5 cm.	11.5 cm.
6.	11.5 cm.	12.0 cm.	7	13.4 cm. and 45°	13.0 cm.	13.4 cm.
7.	11.5 cm.	9.5 cm.	6.5	11.5 cm.	11.0 cm.	11.5 cm.
8.	11.5 cm.	9.5 cm.	6.5	11.5 cm.	12.0 cm.	12.0 cm.
9.	10.0 cm.	9.0 cm.	6.5	10.5 cm.	10.0 cm.	11.0 cm.

* Numbers refer to the distance between primary and secondary coil of a Harvard inductorium and indicate the threshold for pupillary reflex dilatation and stimulation of the sciatic.

table that in 8 out of 9 experiments the threshold of the reflex pupillary dilatation increases under the influence of oxygen concentrations varying from 6.5 to 8 per cent. On readmission of air approximately the same threshold was observed as prior to the administration of the oxygen-poor gas mixture. The table gives, also, the results of experiments in which the same oxygen concentration was used together with 4–5 per cent CO_2. This experi-

ment was carried out immediately after the first anoxia experiment and was followed by another control in which the pupillary reflex threshold was again determined while the animal inhaled air. Under these conditions (see Table 1)

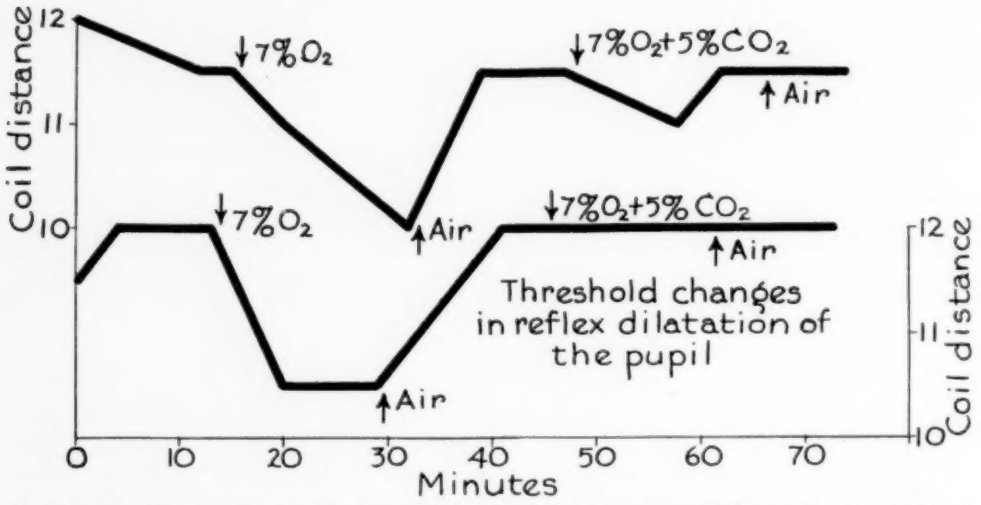

FIG. 1. The effect of 7 per cent O_2 and 7 per cent O_2 +5 per cent CO_2 on the threshold of the sciatic nerve for reflex dilatation of the pupil in the rabbit.

the reduced oxygen tension was either without any effect on the reflex threshold, or the reflex threshold was only slightly increased. A graphic record of the 2 types of response is given in Fig. 1. It is evident that CO_2

Table 2. Unilaterally sympathectomized rabbits.

No. of expt.	Air control threshold	Threshold during anoxia O_2-lack	O_2 conc. per cent	Air control threshold	Threshold during O_2-lack plus 4 per cent CO_2	Air control threshold
1.	8.5 cm.*	6.0 cm.	6.0	7.5 cm.	—	—
2.	8.5 cm.	6.0 cm.	6.0	9.5 cm.	—	—
3.	11.0 cm.	10.5 cm.	6.0	11.0 cm.	—	—
4.	9.5 cm.	8.0 cm.	6.0	9.5 cm.	—	—
5.	8.5 cm.	6.0 cm.	6.0	8.5 cm.	8.0 cm.	8.0 cm.
6.	8.5 cm.	6.0 cm.	6.0	9.5 cm.	9.5 cm.	9.0 cm.

* All figures refer to the threshold reaction observed on the sympathectomized eye.

either completely or partially offsets the effects of anoxia on the pupillary reflex dilatation.

Table 2 gives the results of experiments on the influence of anoxia on the reflex pupillary dilatation in animals in which the cervical sympathetic had been sectioned on one side. The table shows that the changes in threshold in

the sympathectomized eye under the influence of anoxia are similar to those obtained in the normal eye.

Table 3 gives the detailed record of one experiment in which the pupillary response was noted by two observers simultaneously in the normal and in the sympathectomized eye; there is complete agreement in the reactions. Both the normal and the sympathectomized eye show a gradual increase in threshold with regard to reflex pupillary dilatation during the period of anoxia.

In order to explore the possible role played by the sympathetic system

Table 3. Record of experiment on rabbit with unilateral sympathectomy.

Time	Coil Distance in cm.	Right eye*		Result§	Left Eye	
		D_1†	D_2‡			
10:09	8.0	4	6	+	+	
10:11	9.0	4	4	−	−	Animal inhales air
10:13	8.5	4	6	+	+	
10:15	9.0	4	4	−	−	
10:17	8.5	4	6	+	+	
10:20	8.5	5	5	−	−	
10:22	8.5	5	5	−	−	
10:24	8.0	5	7	+	+	
10:26	8.0	6	7	(+)	(+)	
10:28	8.0	6	6	−	−	Animal inhales 6% O_2
10:30	7.5	6	7	(+)	(+)	
10:32	7.5	6	7	(+)	−	
10:39	7.0	5	8	+		
10:41	7.0	5	6	(+)	−	
10:43	6.5	6	8	+	+	
10:46	7.0	7	8	(+)	−	
11:07	8.0	4	8	+	+	Animal inhales air
11:09	8.5	5	7	+		

* Sympathectomized.
† D_1 = diameter before stimulation, 1 unit = 0.5 mm.
‡ D_2 = diameter after stimulation.
§ + = threshold dilatation = 1.0 mm.
(+) = sub-threshold dilatation = 0.5 mm.
− = no dilatation.

the reactions described, 5 experiments were carried out in which first the threshold for the reflex pupillary dilatation was determined, *i.e.*, between 8 and 10 cm. coil distance. Then a drop of 1 per cent homatropin was placed in the normal eye. This abolished the oculomotor tonus and the light reaction in 30 min., while the sympathectomized eye was unaffected. Stimulation of the sciatic nerve for 30 sec. at 0 cm. caused no further dilatation of the atropinized eye, although evoking distinct pain reactions from the animal. There was also marked exophthalmos in both eyes. The atropinized pupil could then be further dilated by the instillation of adrenalin and cocaine. Thus no activation of the dilator pupillae was found by impulses passing over the cervical sympathetic in response to peripheral stimulation.

In a last series of experiments the relationship of the reactions to adrenalin secretion was investigated. Two experiments may be cited in which, after the establishment of the reflex threshold, the administration of 6 per cent oxygen, in conjunction with urethane, gave such a deep narcosis that the animal did not give any pain response or reflex pupillary dilatation whatever to sciatic stimulation. Absence of the pain reaction permitted stimulation of the sciatic nerve for 90 sec. with the secondary coil at 0 cm. distance. After that time the sympathectomized eye showed a dilatation of three divisions which took about 2 min. to reach its maximal value and was maintained for 4 min. This reaction differs markedly in latency and speed from the pupillary dilatation in all other experiments in which weak stimuli were used. The slow reaction is obviously due to a humoral excitation of the dilator of the pupil, since the dilatation involving inhibition of the parasympathetic was eliminated by the influence of the narcosis and anoxia.

Discussion

The experiments reported in this paper give conclusive evidence that under the influence of 6–8 per cent oxygen applied for a period of 20 min., the threshold for the pupillary reflex dilatation increases. Furthermore, it was shown that 4–5 per cent CO_2 inhaled simultaneously with a gas with a low oxygen tension offsets to a large extent, or completely, the effects of oxygen lack on the subcortical centers involved. The results are strictly comparable to our observations in humans on the coloric nystagmus and to the experiments on rabbits involving galvanic nystagmus in narcosis. Under the conditions of these experiments in which weak stimuli were given for short periods of time (1–3 sec.) the reaction studied seemed to involve nothing but inhibition of the parasympathetic. This is proved by the fact that the reaction is quantitatively the same in the normal and in the sympathectomized eye. It is furthermore supported by the fact that the stimuli used promptly evoke a dilatation of the sympathectomized eye but fail to do so in the atropinized eye. That the humoral dilatation caused by the liberation of adrenalin plays no part in these experiments is clear not only from the literature, from which it is evident that adrenalin secretion requires stronger stimuli of longer duration (Cannon and Rapport, 1921), but also from our direct observations in which only excessive stimuli applied for more than 1 min. brought about a humoral dilatation of the pupil. The slow onset of the reaction and the slow return of the pupil to its original size distinguishes this reaction fundamentally from those described in this paper.

We therefore conclude that under the conditions of our experiments the reflex dilatation is due solely to an inhibition of the parasympathetic tone of the eye. Summarizing the experiments here described with those reported earlier it may be stated that anoxia diminishes both excitatory and inhibitory processes in the central nervous system.

Summary

Experiments are reported in rabbits on the influence of stimulation of the sciatic with weak faradic currents on the reflex pupillary dilatation. Six to eight per cent oxygen inhaled for a period of 20 min. increases considerably the threshold for the reflex dilatation. The reaction is reversible on administration of air, and is identical in the sympathectomized and normal eye. The simultaneous inhalation of 4 per cent CO_2 prevents the effects of anoxia. An analysis of the conditions of the experiments makes it highly probable that the pupillary reflex studied in this paper involves only the inhibition of the parasympathetic. If this is the case it may be stated that both excitatory and inhibitory processes in the central nervous system are diminished under the influence of anoxia.

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FACTORS CONTROLLING BRAIN POTENTIALS IN THE CAT*

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IT IS NOW ESTABLISHED that central neurones may exhibit spontaneous rhythmic potentials. Even when synaptic transmission is blocked these spontaneous waves continue and may increase in amplitude (Libet and Gerard, 1938). We have studied in the cat some of the physical and chemical factors that influence this "intrinsic" periodicity, as well as the effect on it of external stimulation under varying physico-chemical conditions; certain neural factors affecting the rhythm have also been investigated.

METHOD

The left hemisphere of cats was widely exposed under light nembutal anaesthesia (35 mg. per kg. intraperitoneally), and the Horsley-Clarke instrument attached. An hour later a concentric needle was placed in the desired position along the optic pathways and amplified potentials recorded with a crystograph or cathode ray oscillograph. Retinal stimulation was caused usually by a flash-light directed into the contralateral eye, or sometimes by a single flash of a condenser discharge through a 2.5 V. bulb. A polarizing current (less than 1.0 mA.) was passed through brain tissue between an indifferent silver plate (2 to 3 cm. surface) applied to the surface of the opposite cerebral cortex, and a silver wire (2 to 3 mm. uninsulated tip) placed 3 to 4 mm. lateral to the recording electrode. Solutions injected into the carotid artery or femoral vein were isotonic, neutral, and at body temperature; and those applied to the cortex directly were often buffered with phosphate (this did not alter their action, although it increased control potentials). The various procedures were carried out in irregular order over a period of 10 to 12 hr. Additional anesthetic was sometimes needed towards the end of the experiment. Each agent was tested in not less than 4, usually in 7 to 12 experiments.

RESULTS

Neural factors

Evoked and spontaneous potentials (normal). The responses evoked by directed retinal stimulation (contralateral eye) vary in form and amplitude with the position of the leading-off electrodes, intensity of stimulus, and extent of previous "dark-adaptation." There is also a cyclic fluctuation in threshold, especially after repetitive stimulation (Bishop, 1933). Threshold, latency, and form have been studied by Bartley (1934), Wang (1934), and by Gerard, Marshall, and Saul (1936), and the present description is intended as a norm with which to compare changes. The geniculate "on" response consists of an initial negative potential (50 to 90 μV.) followed by a prolonged positive wave (Fig. 1B, 2 and 3A). Superimposed upon the latter are often additional positive waves which increase with heightened general excitation (Fig. 4A). The "off" is similar in form and direction, but 20 to 50 μV. less in amplitude. Repeated illuminations evoke in the geniculate, as

* Preliminary reports of this work have appeared in: *Cold Spr. Harb. Mongr.*, 1936, *4:* 292; *Trans. Amer. neurol. Ass.*, 1936, *62:* 55–60, and *Amer. J. Physiol.*, 1938, *123:* 56–57.

in the radiations and cortex (Bartley, 1936), responses which show a semi-rhythmic fluctuation in amplitude and form. "Off" potentials tend to increase on repetition, and the "on" spike may give way to several positive waves. When spontaneous potentials are marked, the responses to light are not discernible, although 1 sec. later they may be strong (Fig. 1Ac).

Throughout the optic pathways a regular rhythm of from 2 to 4 per sec. is present, which usually becomes asynchronous during retinal stimula-

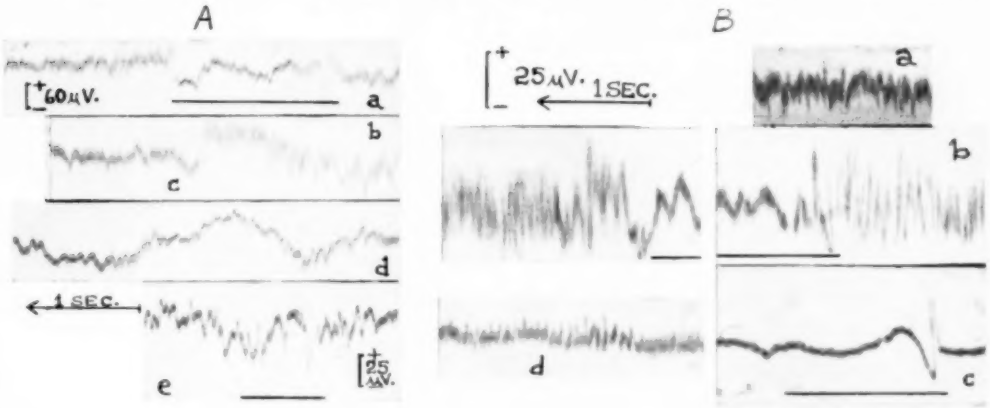

FIG. 1A. Spontaneous and evoked potentials from the lateral geniculate body. After two hours in dark: (a) Response to illumination (black line). Note the initial increase and later suppression of the rapid waves present before illumination; (b) 5 sec. later, note the rhythm at 4.2 a sec.; (c) 10 sec. after (b), rhythm now 2.7 a sec.; (d) moderate diffuse constant illumination, note alternate periods with and without rapid waves; (e) electrode 3 mm. higher, usual "on" and "off" optic response.

FIG. 1B. Lateral geniculate potentials following brain section: (a) Following ipsilateral decortication; (b) 5 min. after subsequent midbrain transection, note greatly increased fast and slow waves, much reduced during illumination; (c) 4 min. after additional nembutal, optic stimulus; (d) 1 hr. after (c).

tion (Gerard, Marshall and Saul, 1936). This rhythm is most prominent with optimum afferent excitation, and was best studied by continuous observations for 6 to 10 hr. at a given locus. After 1 to 2 hr. of absolute darkness, the 2 to 4 per sec. rhythm was often replaced by continuous, irregular, fairly rapid waves. A directed light then gave "on" and "off" responses and inhibited the high frequency, but it did not initiate a rhythm. In diffuse light of moderate intensity (ceiling light on), however, the rhythm reappeared in 4 to 6 sec. This rhythm could be abolished by retinal stimulation with focussed light. The slow rhythm has superimposed on it waves at 8, 20 to 25, and 60 to 80 per sec., all of which are inhibited during light. The 8 and 20 per sec. waves are present only in the lateral geniculate and radiations, and are normally small, 15 and 5 μV. respectively. They are considerably augmented by increased blood potassium, by strychnine and polarization, and during the after-discharge from retinal stimulation. The 60 per sec. waves of 5 to 10 μV. are most prominent in the large ventral cells of the lateral geniculate and fade off in the optic tract and radiations. They persist

after ipsilateral decortication and are markedly augmented following midbrain transection (Fig. 1B), though still eliminated by light directed especially into the contralateral eye. Only under optimal conditions are the slow and the high frequency rhythms distinct in the distal radiations and cortex.

Following a retinal stimulus and the "off" response, the after-discharge modifies for some time the continuous activity. This effect varies with the previous degree of dark adaptation and the number of preceding flashes of light. After a single flash the 3 and 60 per sec. geniculate rhythms return within 5 sec., but following a second flash a high frequency after-discharge develops which lasts 15 to 20 sec. When the slow rhythm returns, it may be half again as fast as normal, *e.g.*, 4.2 per sec. 5 sec. after "off," and 2.7 after another 10 sec. Repeated stimuli (under light anesthesia) induce a regular alternation, at about 1 sec. intervals, with the 60 per sec. rhythm now absent, now present, on the continuing slow waves. This alternation lasts longer after many flashes than after few, but it finally returns to the initial resting state with both rhythms present. Under deeper narcosis, a similar alternation may appear during continuous diffuse illumination of moderate intensity (Fig. 1A a to d), to be inhibited by light directed into the eyes. In the optic tracts, in contrast to the geniculate, the slow rhythm returns almost immediately even after repeated light flashes.

Potentials following section of the brain. Bishop (1933, 1936) attributed the slow rhythm of the optic pathways to reverberating thalamo-cortico-thalamic circuits (Lorente de Nó, 1934, 1935). Interruption of these pathways by uni- or by bilaterial removal of the parietal and occipital cortex does not abolish the geniculate rhythm in the cat (Fig. 1Ba). Complete midbrain transection (Fig. 1Bb), caudal to the colliculi, regularizes the slow rhythm and doubles its amplitude, to 40 μV., and strikingly augments the the 25 per sec. waves, to 25 μV. These modified rhythms persist for hours; are completely inhibited by light directed into the eyes, and return following it; and they are depressed to less than one-fourth their amplitude within 1 min. of the injection of additional nembutal (15 mg. per kg.), gradually to regain full size as the anesthetic wears off.

Physical factors

Brain polarization. A feeble constant current passed through the brain and concentrated toward a "different" needle electrode, placed just lateral to the pick-up electrode, enormously increases the amplitude of potentials in the optic pathways, especially the lateral geniculate, usually with no change in rate (Fig. 2). The direction of the polarizing current is immaterial, although perhaps the different electrode as anode favored the appearance of waves of high frequency. The augmentation may persist for 10 min. following a 30 sec. polarization. Besides the great increase in the slow geniculate rhythm, the "on" and "off" responses to light are also intensified and sometimes are followed by high frequency discharges. The possibility that these changes are an artefact due to the polarizing current is excluded by

the following observations: the induced increase in the rhythm depends on the brain structure and is very marked only in the geniculate, the effects long outlast the polarization, and at a given locus polarization may slightly or greatly increase the rhythm, depending on the state of the animal and other physiological variables.

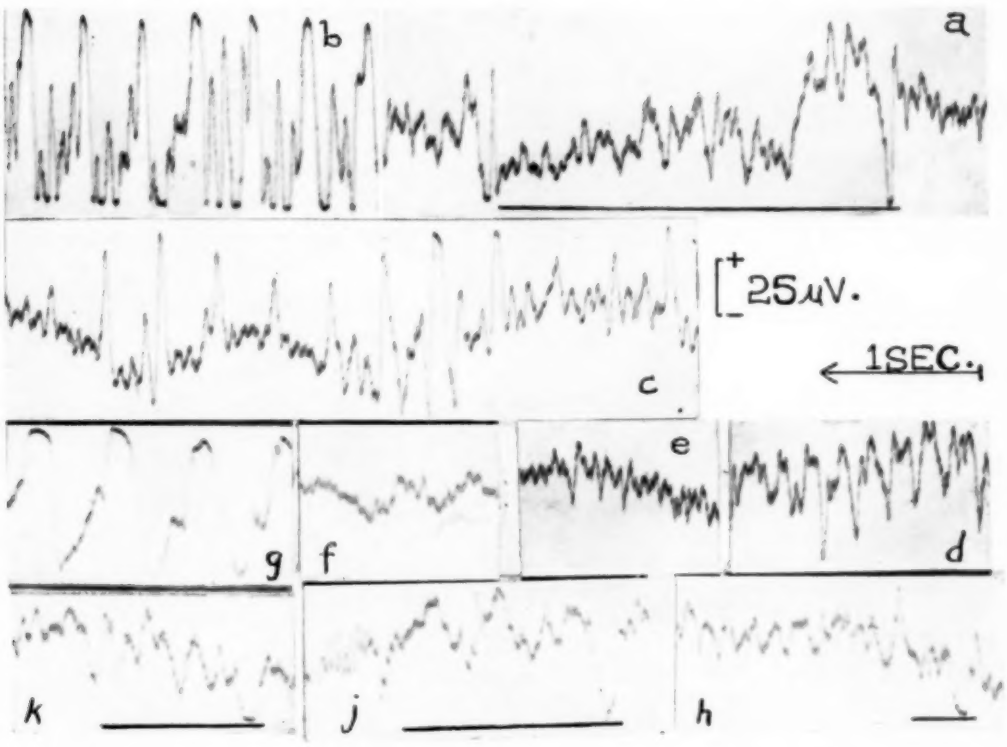

FIG. 2. Potentials modified by brain polarization. Lateral geniculate: (a) Control with light response; (b) during polarization, amplifier overloaded; (c) 10 sec. after polarization; (d) and (e) 2.5 and 13 min. after polarization, respectively. Another cat: (f) control; (g) during polarization. Electrode to optic radiations; (h) control and optic response; (j) during polarization, note rapid waves at "off"; and (k) 10 sec. after polarization.

Chemical factors

Potassium ion. Intravascular injection of isotonic KCl, sufficient to increase the blood potassium from 20 to 50 per cent above normal, induced psychomotor excitation with: gasping respiration, increased deep reflexes, occasional bilateral running movements, urination, loud vocalization, and purposeful scratching gestures. Within 2 min., the slow geniculate rhythm and, especially, the superposed 8 per sec. waves were augmented. When these had again diminished, a retinal stimulus still increased them; 5 min. after injection a continued retinal stimulus gave a somewhat increased "on" response followed by regular 45 per sec. potentials which waxed and waned at 2 per sec. (the frequency of the usual slow rhythm; Fig. 3A). Potentials

of the optic cortex were not clearly affected. Within 10 min. the animal returned to surgical narcosis and the thalamic potentials resumed their pre-injection character. These time relations parallel those of rise in blood po-

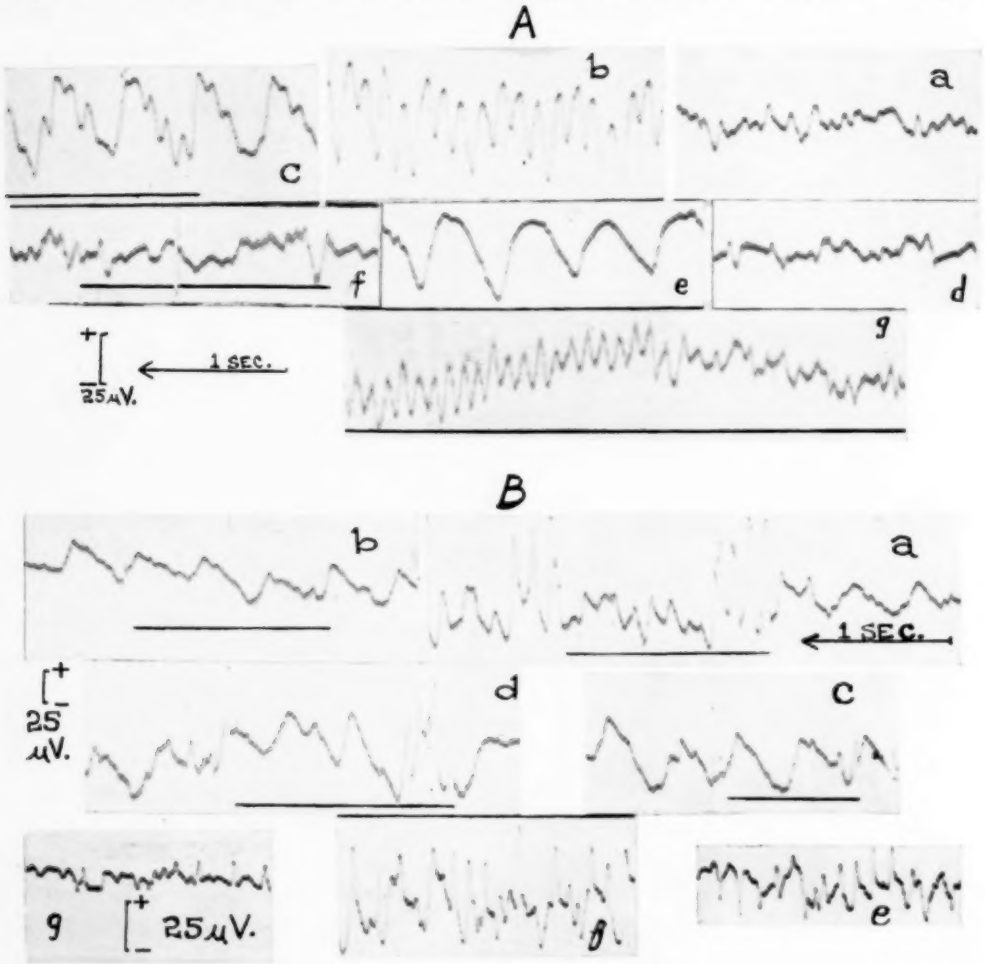

FIG. 3A. Lateral geniculate potentials as modified by ions and strychnine on intravascular injection. (a) Ringer's solution injected as control; (b) 2 min. after K; (c) 30 sec. after (b); (d) 30 min. later; (e) 2 min. after Ca; (f) 15 min. after (e); (g) 4 min. after strychnine.

FIG. 3B. Striate cortex potentials (surface record) as modified by ions locally: (a) Normal rhythm and optic response; (b) 10 min. after K; (c) 10 min. after (b) and washing with Ringer's solution, note limited recovery; (d) 1 min. after (c) and after washing with Ca. Another animal: (e) 10 min. after K; (f) 15 min. after (e) and after washing with Ringer's solution; (g) 2 min. after Ca.

tassium following intravenous potassium administration (Houssay and Marenzi, 1937). The largest single intravenous dose of KCl tolerated was 15 mg. per kg. in cats; Houssay and Marenzi found 20 mg. per kg. in dogs. Death was apparently due to cardiac failure.

Local application of excess K ion to the striate cortex reversibly depresses and may completely abolish spontaneous and evoked potentials. (With sufficiently weak potassium solutions, the expected increase of potentials is obtained.) Ringer's solution with tripled K ion cuts the amplitude to half in 2 min. with spontaneous recovery in 7 to 10 min. Isotonic KCl completely abolishes the normal "on" and "off" potentials (initially 100 μV.) by 2 to 10 min. after application (Fig. 3Ba and b). There is little return in another 10 min. even after washing with warm Ringer's solution (c); but application of isotonic $CaCl_2$ restores normal responses within a minute (d). The spontaneous rhythms are similarly suppressed by potassium excess and restored by calcium. Potassium may also reverse the polarity of the waves (Fig. 3Be).

Calcium ion. Intravascular injection of isotonic $CaCl_2$, sufficient to increase the blood concentration 50 to 200 per cent, depresses the animal, if under light anesthesia. Within 2 min. the fast irregular geniculate potentials disappear, the 2 per sec. rhythm is regularized and enhanced, and the amplitude and duration of the after-discharge to light is decreased (Fig. 3Ad and e). The increased and stabilized slow rhythm now persists during optic stimulation, which normally disrupts it. Intravascular Ca ion excess was found to be less toxic than K ion excess, and calcium action could be tested within 20 min. of a preceding potassium injection.

Local application to the optic cortex of Ringer's solution with three times the normal calcium does not alter potentials, but isotonic $CaCl_2$ depresses evoked and spontaneous ones during 10 min. (Fig. 3Bf and g). The calcium depression is antagonized by potassium or by citrate, which promptly restores normal responses. Calcium ion deficiency, produced by intravascular injection of 2.0 cc. of 3.0 per cent Na citrate in Ringer's solution, acts upon lateral geniculate potentials much like potassium excess.

Hydrogen ion. Intravascular injection during 30 sec., of 1.0 cc. per kg. of 0.01 *N* HCl in Ringer's solution increases respiratory depth and evokes general restlessness and movement. It also disrupts the slow lateral geniculate rhythm, decreases the amplitude of spontaneous and evoked potentials, and prolongs the after-discharge. The rhythm does not reappear for 20 to 30 min. Local application of approximately 0.001 *N* HCl in Ringer's solution (unbuffered) evokes, within 2 min., wave trains at 7 to 12 per sec. and 40 to 60 μV. These reappear during retinal stimulation after the initial effect has worn off.

An *increase* of pH is more toxic than a decrease, but less prolonged in action. Rapid injection of 2 cc. of 0.01 *N* acid or 1.5 cc. of 0.001 *N* alkali per kg. is usually fatal. (Barbitalized dogs can tolerate as much as 3 cc. of *N* HCl injected slowly during 3 min., Harkins and Hastings, 1931; the maximal pH fall, to 6.92, occurs in 6 min.) Smaller amounts of NaOH depress respiration and general motor activity, immediately disrupt the slow geniculate rhythm, and markedly diminish the spontaneous and evoked poten-

tials. Normal potentials return in 6 to 8 min. At these pH extremes, acid and alkali injections do not consistently counteract one another.

Lactate ion. Less than 2 min. after the intravascular injection of 2.0 cc. of 4 per cent Na lactate, the slow geniculate rhythm is less regular and smaller and is largely replaced by rapid, large, irregular potentials. These are especially prominent in the after-discharge following optic stimulation. The modified potentials persist for 1 to 2 hr.

Strychnine. Within 1 min. after the intravascular injection of 2.0 cc. of 1.0 per cent strychnine solution, the slow geniculate rhythm is augmented; often, when no obvious rhythm is present in a dark-adapted cat, strychnine initiates conspicuous rhythmic potentials. During optic stimulation the augmented slow rhythm is replaced by waves at 13 per sec. and 15 μV., to reappear almost immediately after the "off" (Fig. 3Af and g). When some minutes have elapsed following the strychnine injection, the rapid rhythm appears during illumination and continues as an after-discharge. The high frequency potentials of potassium excess, in contrast, appear primarily during light and less frequently in the after-discharge.

Blood sugar. Nembutal anesthesia causes no change in mean blood sugar level (Hrubetz and Blackberg, 1938). Insulin, in a dose of 10 to 15 units per kg. subcutaneously, does not lead to gross convulsions, though geniculate potential changes are definite within an hour. (Compare the time course of blood sugar changes; Zucker and Berg, 1937. Smaller insulin injections do not affect potentials.) The background potentials become augmented and the normal diphasic "on" and "off" responses to light are replaced by 3 to 5 large oscillations, mainly positive, followed by a prolonged high-frequency after-discharge (Fig. 4A). The picture is similar to that induced by potassium excess. Similar quantities of insulin, given intravenously, lead to repetitive optic responses, but they markedly decrease the spontaneous potentials (Fig. 4B). In both cases, the altered responses to light remain constant with repeated stimuli. The normal picture is restored within 5 min. by intravascular glucose (2 cc. of a 40 per cent solution). Hyperglycaemia leads after some 10 min. to an increase in rate and amplitude of the slow rhythm (Fig. 4Ag and h).

Hypoxia. Bilateral occlusion, by traction, of the carotid arteries reversibly abolishes the spontaneous and evoked potentials in the lateral geniculate body. Electrical activity disappears usually in about 1 min., and returns in 1 to 3 min. after release. Optic responses are lost at the same time as the spontaneous ones. In 3 animals with transected brain stem (and loss of vertebral blood) the average times of loss and return of potential were 50 sec. and 80 sec., as compared with 240 and 400 sec. in 3 intact animals. Before anoxic failure and early during subsequent recovery, potentials are increased, with a large high-frequency component.

Discussion

The unevoked potential rhythms of central neurones are controlled by their own states of metabolism, membrane charge, etc., which in turn are

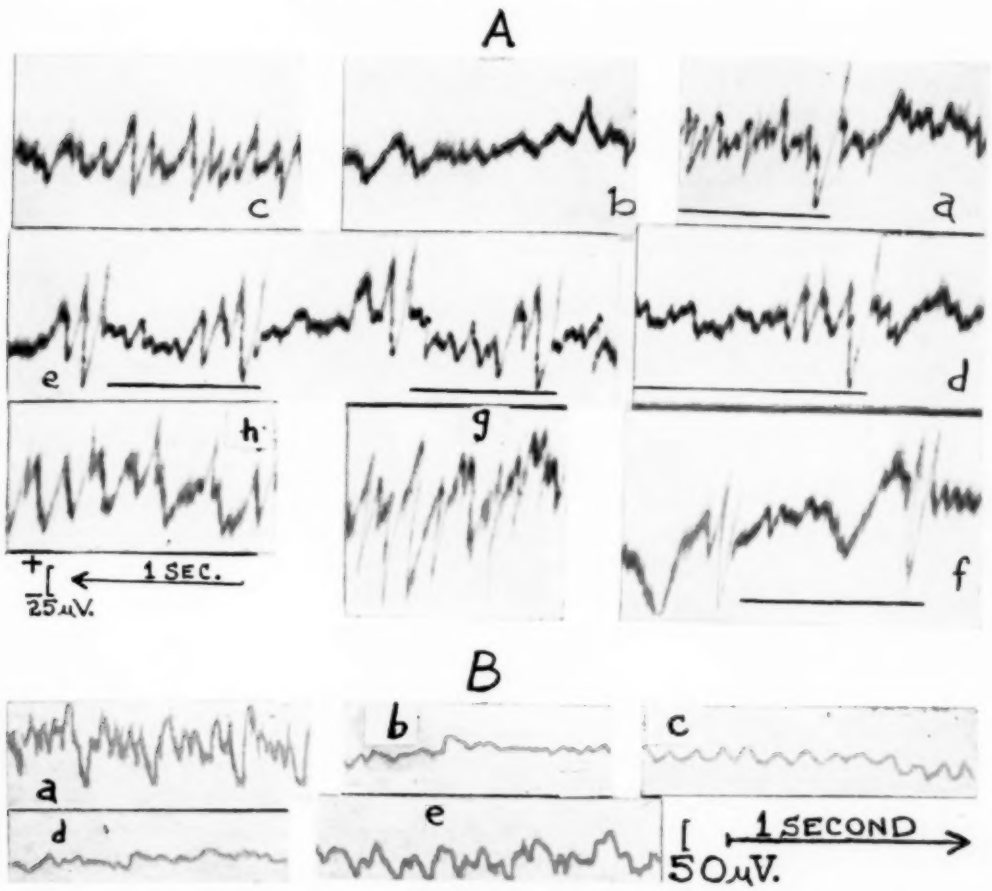

FIG. 4A. Lateral geniculate potentials as altered by change in blood sugar: (a) Normal "on"; (b) 2 sec. and (c) 10 sec. after "off"; (d) 20 min. after insulin (10 units subcut.) single "on" and (e) repeated optic stimuli, note multiple waves; (f) 6 min. after (e) and intravenous glucose, "on" and "off"; (g) 2 and (h) 20 sec. after (f).

FIG. 4B. Like Figure 4A, undulator ink records obtained at Ohio State University. (a) Before insulin; (b) 70 min. after intravenous insulin, dark, (c) during light, (d) dark, 2 min. later; (e) 8 min. later, following intravenous glucose.

modified by influent nerve inpulses and the condition of the surrounding medium. As recorded, these potentials involve the further factors of the number, kind, and distribution of active cells or cell groups and the degree of unison of their separate beats. Interpretation of such records at present remains rather circumstantial for the mammalian brain. Cells of the lateral geniculate nucleus are roughly stratified with the largest ones located most ventrally. Of the four more or less distinct rhythms, the faster are more prominent in the ventral portion. Perhaps the larger cells normally manifest a faster rhythm, but at all needle positions there is considerable play of potential rate from time to time under seemingly fixed conditions.

The slow 3 per sec. rhythm, seen especially in the geniculate, might arise in the eye and be normally imposed on other portions of the optic system.

It is seen in the primary tract and, after abolition by light, reappears in it earlier than in the geniculate. On the other hand, the geniculate rhythm persists for some hours after severing the optic nerves, so that the thalamic cells can maintain the beat alone. It similarly survives, is even enhanced by, the removal of the optic cortex, so that any thalamo-cortical cycle which may exist is not essential. The same is true for connections with the mesencephalon, section of them favors the diencephalic rhythm (see also Bremer, 1935), and even full synaptic blockade with nicotine leaves the geniculate active for a time (Libet and Gerard, 1938). These neurones, then, do not depend on immediate nerve impulses from eye, cortex, or brain stem to maintain their beat.

Over longer periods this may not be true; also, at any given moment, the local behavior is modifiable by incoming impulses. The absence of the rhythm after prolonged dark and its return in moderate diffuse light can be interpreted in terms of a state of cell excitation which slowly falls below threshold for the rhythm when no afferent impulses are being received. Any enhancement of the general level of cell excitation (whatever the metabolic, membrane, or other mechanism for controlling this level may be) favors the potential waves; and they are increased during diffuse and after strong optic stimulation and by potassium and strychnine.

The disruption of the rhythm during strong light has been generally interpreted as a desynchronization ot the many cells, rather than as a decrease in the beat of each. The effect of polarization is presumably just the reverse of this. A rhythm, initially feeble and more or less obscured by the partial asynchrony of cells, is greatly increased and regularized (but not altered in form or rate), as an imposed potential sweeps the cells into unison. The change is independent of the direction of the imposed current, which in any event flows across irregularly oriented cell processes, and outlasts the current for many minutes. The current may, of course, alter the beat of individual cells as well as their phase relations. On this there is no evidence except that a cell stimulation would probably increase rate, which occurred in only one case.

The influence of potassium increase and calcium increase or decrease (citrate), on optic potentials is in accord with their action on neurone metabolism and activity (see Gerard, 1938). Moderate increase of potassium enhances irritability and excitation, and it augments potentials, especially the faster rhythms. Greater concentrations of potassium, applied locally to the cortex, depress irritability and potentials. Calcium decrease acts similarly, excess oppositely, and increase of both potassium and calcium antagonizes the action of excess of either alone. After-discharge following optic stimulation is prolonged by potassium, shortened by calcium. (Cf. motor discharge after cerebellar stimulation; Gerard and Magoun, 1937.) The dominant 3 per sec. wave of the geniculate is not abolished by calcium injections but, due to suppression of faster waves, remains more clear than normal. It may be actually strengthened, or at least cell synchrony made more positive, by increased calcium since light no longer disrupts it. Possibly

cells, which at "normal" excitation levels produce the faster rhythms, fall into the slower tempo when somewhat depressed by calcium.

The marked increase and decrease of pH studied here both disrupt the slow rhythm. Acidity increases the 8 per sec. waves and prolongs after-discharge, alkalinity depresses all waves, including evoked potentials and their after-discharge. Over a finer range, cortical potentials are increased by an alkaline shift in the cortex and diminished by an acid one (Dusser de Barenne, McCulloch, and Nims, 1937), in parallel to changes in irritability. The action of strychnine (by injection), in initiating or increasing a regular slow rhythm and in making more rapid and intense the after-discharge following physiological stimulation, requires no comment. Strychnine spikes were not seen in any of these experiments as on local application. (See Jasper, 1937; Dusser de Barenne and McCulloch, 1938.)

Of especial interest are the effects of hypoglycaemia (insulin) and of glucose injection. Others have reported, for the cortex, mainly of human subjects, a slowing of the normal rhythms under insulin action (Hoagland, Rubin and Cameron, 1937; Lennox, Gibbs, and Gibbs, 1938). The "delta index," of slow wave components, inversely follows blood sugar concentration, sometimes quite accurately. Such slowing has been interpreted as evidence of a decreased metabolism of the active neurones. On the other hand, hypoglycaemia acts much like hypoxia, which induces increased neural sensitivity and discharge as a transient phase between the normal and depressed states. (Gerard, 1938.) The present findings for the geniculate indicate such an excitation. Under insulin action (and promptly abolished by glucose) the spontaneous rhythm is enhanced, the "on" and "off" light responses are larger than and show complex potential swings not present in the normal ones, and the after-discharge is more prolonged and of a higher frequency. Hypoglycaemia, in fact, affects geniculate potentials much as does increased blood potassium—even to a reversal of the excitant action when concentration changes are excessive. Insulin causes a decrease in blood potassium (Keyes, 1938) while anoxia causes an increase (Mullin, Dennis and Calvin, 1938). In the latter case the potassium content of the brain is, if anything, increased (Gerard and Tupikova, unpublished), the potassium entering into circulation from other sources. Under insulin, also, it is possible that brain potassium is increased, some entering from the blood. This has yet to be determined. By whatever means low blood or brain carbohydrate produces its action, it does seem that it is able to increase brain activity as well as to depress it.

Summary

The normal spontaneous rhythms of the cat's geniculate body, especially the dominant one at 3 per sec., are independent of impulses reaching these neurones from optic nerves, cortex or brain stem. The background level of excitation, determined largely by optic impulses, however, strongly influences their character. The slow rhythm fades out over hours in the dark and is reinitiated after brief illumination.

The enhanced spontaneous and evoked optic potentials induced by potassium, citrate, acid, strychnine, insulin and polarizing currents, and the diminished potentials resulting from calcium, alkali and glucose are described and interpreted.

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CONTROL OF THE POTENTIAL RHYTHM OF THE ISOLATED FROG BRAIN*

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INTRODUCTION

THE PRESENCE of "spontaneous" rhythmic electric potentials in groups of nerve cells has been observed by most students of electrophysiology. These potentials exist in the absence of deliberately evoked nervous impulses (Berger, 1929; Bartley and Bishop, 1933; Gerard, Marshall and Saul, 1936; and others) and they persist, even for hours (Gerard and Young, 1937), in isolated groups of nerve cells (Adrian, 1931; Adrian and Buytendijk, 1931) despite the blocking of synaptic conduction by nicotine (Libet and Gerard, 1938). Such facts give impressive evidence that the rhythms are truly spontaneous, in the sense that no nerve impulses are immediately necessary to their evocation.

Several laboratories have investigated the physico-chemical factors influencing these rhythms in man and laboratory mammals (Dubner and Gerard, 1936 and 1939; Hoagland, 1936; Hoagland *et al.*, 1937; Lennox *et al.*, 1938; Jasper, 1936; Davis, 1936; see also Prosser, 1933, on the crayfish). The present experiments, directed to the same end, have been carried out under the much simpler conditions offered by the isolated frog brain. Information has also accumulated on the influence of incoming nerve impulses on cell potentials and concerning the mechanisms of synchronization of cell groups.

METHODS

Preparation. The brains of grass frogs (*R. pipiens* and *R. foxinus*) and bullfrogs (*R. catesbiana*) were used. Results were similar for all, freshly captured animals yielding the best preparations. Potentials from the brains of bullfrogs average perhaps twice the amplitude of those of the smaller frogs, although some of the latter have shown the greatest activity; and bullfrogs were used for experiments on the olfactory nerves because of their larger structures. The brain can be exposed and removed either from the separated head (decapitation without anesthesia) or from the intact etherized animal. Decapitation is accomplished by a single snip of a pair of scissors at the angles of the mouth, with the top blade as far caudalward as possible. The dorsal skin is reflected and, with the eye and muscles, trimmed off on one side. The skull is rapidly twisted outward with sturdy forceps from the open rear end forward, the brain gently lifted to one side, and the cranial nerves, including the olfactory, cut through. The freed brain is lifted with a small spatula or forceps and placed in Ringer's solution in a small glass container. The dura mater often slides off as the brain is lifted with forceps; if not it is carefully cut away. Pressure or tension, especially on the olfactory bulbs, must be carefully avoided. The original decapitation must be by shearing rather than crushing and the olfactory nerves severed (with fine scissors slipped forward under the brain) before lifting the brain. After some practice, this whole procedure requires less than 2 min. and the isolated preparation manifests potential rhythms almost without exception. This procedure is not applicable to the bullfrog, with tougher bony plates, and the brain is exposed in the usual manner in the etherized animal. It is then covered with cotton soaked in Ringer's solution and the frog allowed to

* A preliminary report of this work appeared in *Amer. J. Physiol.*, 1938, *123:* 128.

blow off the ether for 1.5 to 2 hr.; after which time the dura is reflected, the brain sectioned in front of the optic lobes, the olfactory, optic and oculomotor nerves cut, and the forepiece of brain removed to the container. Grass frog brains give like results when removed by decapitation or after etherization. Occasionally, recordings were made from the exposed intact brain *in situ* with the animal immobilized.

Electrodes. Ag–AgCl-wick electrodes (Adrian, 1936) were used. Silver tubes (0.3 mm. internal diameter, 15 mm. long), attached to solder wires for separate adjustment of each electrode, were held in a clamp with universal adjustment. A Ringer-soaked cotton thread, 0.2 mm. in diameter and projecting 1 to 2 mm. from the tube, served as wick. With this in place, a current of 1.0 mA. for 2 hr. deposited AgCl between tube and thread giving an electrode of some 10,000 Ω. resistance. During 3 or 4 weeks, although stored in Ringer's solution, resistance rises to 30,000 Ω, when the electrode is discarded. One wick was regularly placed on an olfactory bulb, the other 10 mm. lateral in Ringer's solution (or on bone). During the recording, the solution was sucked away so that only a film connected brain and indifferent electrode. It was found possible repeatedly to reset electrodes and to replace and remove solution with no significant change in the amplitude of recorded potentials; so that physical short-circuiting was constant and physiological injury absent. The

Table 1.

mM/liter	Frog plasma (Fenn)	Ringer used
K	2.5	1.9
Na	103.8	111.0
Mg	3.0	—
Ca	2.0	1.1
Cl	74.3	114.0
HCO_3	25.4	—
Lactate	3.3	—
Phosphate	3.1	—
	215	228

electrode was lowered just until the fluid on the brain and wick coalesced, for pressure soon disrupted the slow rhythm. Grounding the indifferent lead had no effect. Fine platinum electrodes (0.3 mm.) were sometimes used, with one or both placed on or in the olfactory bulb. These yielded larger potentials but less regular and less constant ones, due to pressure injury, and were employed only for special purposes.

Recording. The push-pull, resistance-capacity coupled amplifiers, cathode ray oscillograph and crystograph were used according to standard practice in this laboratory. The usual controls of the electrical system, *e.g.*, with electrodes on dead brain, established the validity of observations on the living tissue.

Solutions. The ionic composition of Ringer's solution (Starling, 1936) differed somewhat from that of frog plasma (Fenn, 1936; Table 1). Since addition of carbonate or phosphate in the amounts present in plasma did not affect results, their absence is immaterial. Phosphate buffer (pH 7.4, 1/75 or 1/150 *M*) was also without influence and, therefore, usually omitted when not required. Magnesium, in the concentration normally present, does affect potentials (see below), and its absence from the Ringer's solution may have modified the findings; the higher ratio of K to Ca + Mg in our solution than in plasma perhaps contributing to the final disruption of the rhythm; but the saline solution as used would not have masked the action of the added ions since their effects increased with the concentration and were also reversible. Substances to be tested were added to the Ringer's solution without equivalent removal of ions; the slight increase in osmotic pressure so produced being well below that required to alter potentials. For pH values between 6.0 and 7.8, phosphate buffer was used; below 6.0, acetate; and above 7.8, borate (with NaCl replacing KCl in the buffer). The final pH was checked to 0.02 units with glass or hydrogen electrodes. No calcium precipitated, even after long standing of the buffers.

The solution surrounding the brain was changed by a standard procedure, to favor constant diffusion and other conditions of action of the substance tested. The electrodes

were raised, the solution sucked out, the brain and electrodes washed twice by gentle flooding with new solution and then left covered by it for 3 min.; finally the fluid was sucked out and the electrodes lowered into place. Replacing old Ringer's solution with fresh, to control the procedure, caused no change in potentials.

Temperature. For most experiments, room temperature, 22°C., was sufficiently constant. When studying temperature itself, the brain container was submerged almost to the rim in water in a Dewar flask and the brain nearly covered with saline at the same temperature. One junction of a thermocouple, lagged thermally with paraffin, hung into the water, the other was inserted into a cerebral hemisphere posterior to the bulbs. The water temperature was directly measured to 0.1°C. and did not change this much during a single run. Any difference between the temperature of the brain and the water was measured via the thermocouple to 0.05°C., a single determination requiring less than 10 sec. The actual temperature of the cerebral hemisphere was thus accurately followed as the system was changed from one temperature to another. Since electrical records were obtained from the olfactory bulb, with a wick electrode conducting heat, rather than from the hemisphere, where temperature was measured with 38-gauge wire conductor, other controls (dead brain, electrode and thermocouple together on bulb, etc.) were made to test the temperature comparability. No difference was seen in the rate of attaining temperature equilibrium by the two brain regions. The maximal temperature error possible in these measurements, 0.1°C., would not alter the estimated Q_{10} by 1 per cent.

Stimulation. Olfactory nerves were sometimes stimulated by induction shocks via platinum electrodes fixed in the container. The primary was fed waves at 40 to 60 per sec. from a thyratron. Stimulus artifact could not be eliminated but recording was started in less than 1 sec. after the end of tetanization.

Results

Experiments on over 200 brains were directed to the study of such basic influences as temperature, salt and hydrogen ions, etc., on potentials. Such information is valuable in itself, and is also necessary to the examination of the action of more specific substances to be reported later.

Spontaneous rhythm

In vivo. The exposed uninjured olfactory bulbs of a frog, after recovery from anesthesia, exhibit a basic rhythm which is ordinarily constant in frequency and amplitude in any individual to within 5 per cent, irregularity being partly due to variation in superposed higher frequency waves. The fundamental frequency averages 6.0 per sec., extremes 4.0 and 8.5, with an average amplitude of 30 μV., extremes 10 and 40. The whole surface of the bulb is electrically active. Exploration with a fine electrode (*in situ* and after isolation) shows the greatest and most regular potentials on the free lateral aspect and on the dorso-medial surface; the dorsal aspect between these is less active and the ventral still less. Electrodes on both bulbs record the same potentials as an active electrode on one and an indifferent electrode elsewhere, showing lack of electrical synchrony of the two sides. The faster feebler rhythms encountered in other parts of the brain (Gerard and Young, 1937) have not been further examined.

In vitro. Immediately after isolation of the brain, the olfactory bulb rhythm is much stronger, average 100 μV., and more regular in height and wave form. Its average frequency is not altered but the moment-to-moment variability is decreased, from 5 per cent to 2 (Fig. 1A a and b). This powerful beat continues in the absence of measurable impulses from the olfactory

nerve or the remaining brain; for the former shows no action potentials and the electrical record from the hemisphere is flat in 15 to 20 min. after isolation, that from the optic lobe in 30 min. The bulb itself may continue to

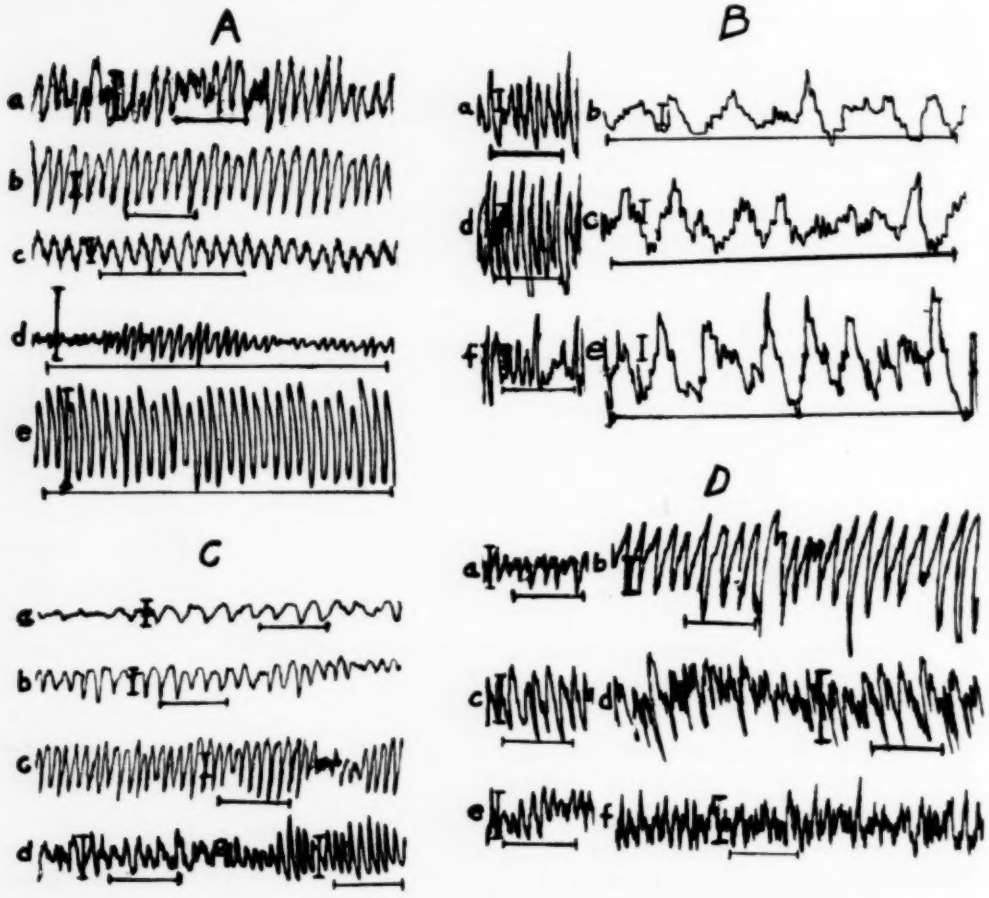

FIG. 1. A. a. Olfactory bulb *in situ*. b. Same bulb 1 min. after isolation of brain. c. Two bulbs separated from each other but attached to hemisphere. d. Single separated bulb. e. Single bulb fragment, about 0.1 mg.

B. a and b. Normal. c. 3 sec. after a 12-sec. stimulation of the olfactory nerves. d, e, f, 4.5, 6.5 and 13 min. after stimulation.

C. Bulb at: a. 15°C.; b. 22°C.; and c. 33°C. Another at: d. 22°C.; e. 28°C.

D. a, c, e, normal controls, each a separate brain; b, d, f. after 5 min. in test solutions. b. Ringer's plus glucose to double osmotic pressure. d. Ringer's plus acetate buffer, pH 5.5. f. Ringer's plus borate buffer, pH 9.0. Electrical records in all figures are from the olfactory bulb of an isolated brain except as indicated. The horizontal straight line represents one second, read left to right, the vertical one, 30 μV, in all cases.

give potentials for 3 hr. or more, even with no special handling. Often after 10 to 15 min. the steady rhythm begins to wax and wane, and after 30 to 60 min. individual waves become somewhat irregular. Irregularity slowly increases and one or more faster rhythms (10 to 45 per sec.) come to supplant

the usual one. Amplitude falls with increased frequency and decreased regularity until the waves finally fade into the baseline. If frog serum is used in place of Ringer's solution, the 6 per sec. rhythm is retained longer (60–90 min.), irregularity is not so marked, and activity is not finally lost until some 4 hr. after isolation.

Further separation of the bulbs, by cutting away the hemispheres with a razor, results in inactivity, due to pressure injury by the blade. Such pressure was avoided by separating the brain tissue with two pairs of finely ground forceps, the tissue not immediately about the instrument thus being spared mechanical insult. In similar fashion, the two bulbs were later separated from each other, and even a single bulb picked to pieces. Each successive step led, in the majority of cases, to a discontinuous increase in frequency. Thus, for example, removing the hemispheres raised the frequency from 6 to 8 per sec.; separating the two bulbs raised it to 10 per sec.; isolating a bulb completely, to 30 to 40 per sec.; and, finally, isolated bulb fragments of about 0.1 mg. gave a regular rhythm, *e.g.*, at 30 per sec. and of 15 μV. intensity (Fig. 1A c, d, e). There was usually a corresponding loss in amplitude, but occasionally an increase occurred in this as well as in the frequency. In two instances, bulbs which had ceased activity regained it after separation from the hemispheres; and, in another case, activity was enhanced at once on separation, and continued to increase further during several minutes.

Effects of stimulation

The return or increase of olfactory bulb potentials produced by isolation procedures must be due to removal of inhibitory control or to direct excitation. The latter could be further tested by electrical stimulation of the olfactory nerves or the bulbs themselves. Tetanizing bulbs which had ceased activity restored spontaneous potentials in 3 of 5 cases. The potentials were feeble and irregular and resembled those usually seen some 20 min. prior to inactivity. When the nerves were stimulated (10 sec. 40 shocks per sec.) in the freshly isolated brain, the regular rhythm was disrupted and replaced by irregular small 30 to 50 per sec. waves. This picture outlasted the stimulation for 2 to 3 sec., when the 6 per sec. rhythm reappeared, to increase in amplitude and regularity during another 2 to 4 min. (Fig. 1B); by this time *i.e.*, 4 to 7 min. after stimulation, the regular rhythm had attained an amplitude 25 per cent greater than before stimulation and the intensity then fell slowly, over an average period of 12 min., until it became normal. More prolonged stimulation caused more enduring post-stimulation changes. In only 2 of 12 experiments was tetanization of the nerve without effect, synaptic transmission perhaps having failed in the 2 cases before the test.

Temperature

The temperature of the isolated brain was varied between 8 and 35°C. but usually only between 14 and 30°C. At lower temperatures potentials gradually flattened to basal level; at higher temperatures, wave amplitude,

after brief but conspicuous increase, fell abruptly and irreversibly. In moderate temperature ranges, amplitude and especially frequency of the rhythm increased with temperature (Fig. 1C). When the temperature was brought to a new level the brain attained the new temperature, as shown by a thermocouple, in 4 to 5 min. The rhythm change lagged behind this and might require 4.5 to 6.5 min. to reach a new steady value (Table 2). In 16

Table 2. Exp. 3/8/38. Bullfrog. Ether at 2:00 p.m. Brain exposed 2:15 p.m., isolated (to middle of cerebral hemisphere) 4:35 p.m. Readings start at 4:50 p.m.

Temp. (Centigrade) around brain	Time (min.)	Brain temp.	Frequency (per sec.)	Q_{10} of frequency	Amplitude (μV.)	Q_{10} of amplitude
23°	0	22.9	7.0		60	
to	to			2.2		
29°	0.25					
	1.0	24.4	7.8	2.5	70	1.9
	1.75	26.0	9.0		75	
	2.5	26.4	9.5	3.6	80	
	3.25	26.9	10.1			
	4.0	26.9	10.1		75	
	4.5					
to	to			2.7		
23.5°	4.75			2.5		1.5
	6.0	24.3	7.8		60	
*	7.6	23.6	7.5		65	
	16.0	23.2	6.0		70	
to	to			1.5		
16°	16.25					
	17.0	20.2	5.5	2.1	65	
	17.3	19.0	5.1		55	2.1
	18.25	17.7	4.0	5.7	55	
	19.4	17.0	3.6		45	
	20.65	16.5	3.4		40	
	23.1	16.2	3.0		40	
to	to			3.7		
23.3°	23.75			3.0		2.7
	25.5	21.5	6.0		80	
	27.75	22.7	6.3		80	

* Q_{10} between 26.9°–16.2° is 3.1, but this high value must be discounted because of a break in the experiment at this point.

experiments in the moderate temperature range, the Q_{10} of frequency fell between 2.1 and 2.6, with an average of 2.3. The Q_{10} for amplitude, although of the same order, was more variable. This was due to greater dependence of amplitude than of frequency on the time of exposure to each temperature and to "spindling" of wave trains, which necessitated the arbitrary use of half the maximal amplitude for the calculations. A typical experiment is shown in Table 2, and four are plotted in Fig. 2 where the Q_{10} is calculated from Belehrádek's (1935) formula:

$$Q_{10} = \left(\frac{K_1}{K_2}\right)^{10/t_1 - t_2}$$

where K_1 and K_2 are the wave magnitudes at the Centrigrade temperatures, t_1 and t_2, respectively. The Q_{10} is greater in the lower temperature range (cf. Gasser, 1931), and the coefficient tends to be greater for a given temperature step on passing from the lower to the higher value than vice versa. Besides thermal control of frequency and amplitude, the regularity of the waves consistently improves, with a rise in temperature, while cooling al-

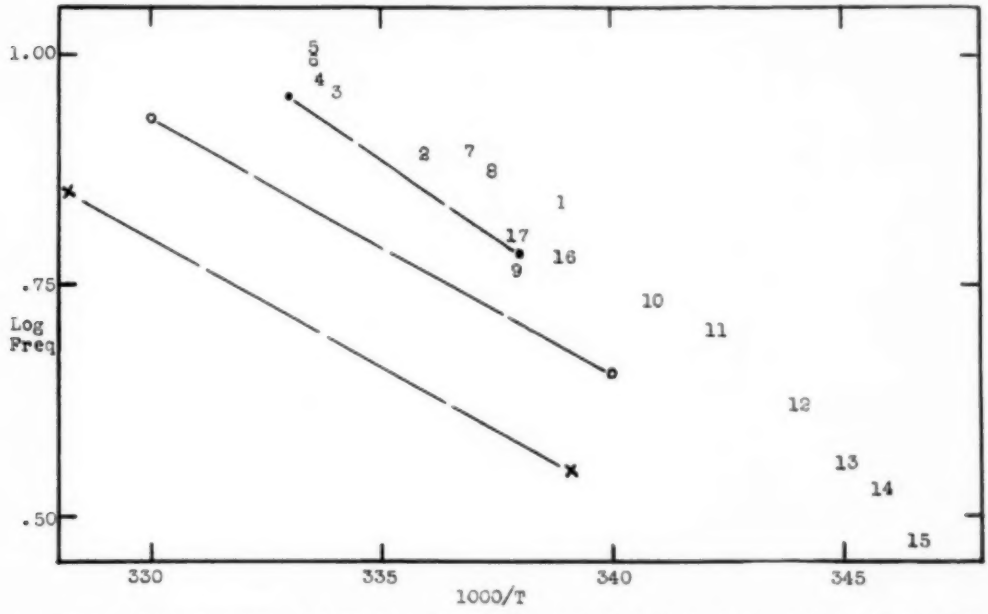

FIG. 2. Log. frequency plotted against reciprocal of absolute temperature. ×, 0 and • data from 3 separate brains. The numerals are the observations of Table 2, the number giving the order in which observations were made, plotted to these coordinates.

ways makes the wave shape more irregular (Fig. 1C). Greater regularity means a better alignment of activity in time, regardless of what components make up the wave. Consequently the process of synchronization is facilitated by a rise in temperature.

Osmotic pressure

Addition of sucrose or glucose to the Ringer's solution, sufficient to increase osmotic pressure by 50 per cent, does not affect potentials. Doubling the osmotic pressure with either sugar caused a change after 3 min. soaking in 4 of 9 experiments. The waves slowed 15 to 20 per cent, *e.g.*, from 6.1 to 4.9 per sec., increased in regularity, and became 10 to 20 per cent larger (Fig. 1D a, b). The same osmotic change produced by doubling the Ringer salts, in 3 experiments, caused marked irregularity with the appearance of frequencies of 10 to 40 per sec. and a great loss of amplitude, not due solely to increased conductivity. Diluting Ringer's solution with an equal volume of water lowered the normal frequency only 10 per cent and made

the waves rather more regular; their amplitude was increased by half, after correcting for resistance changes. In water, potentials soon became irregular and disappeared after about 15 min. Most of the effects resulting from altered salt concentration depend more on specific ion changes than on changes in osmotic pressure.

H-ion concentration

Soaking in 0.013 *M* phosphate buffer has no effect on potentials. At pH values below 6.2 or above 7.8 changes are seen after 5 min. soaking. In acid solutions (acetate buffer) the 6 per sec. rhythm slows discontinuously to about 4 per sec. and the waves become less regular, with superposed frequencies of 15 to 40 per sec. (Fig. 1D c, d). In alkaline solutions (borate buffer), the normal rhythm became irregular and fairly strong waves at 15 to 30 per sec. are added (Fig. 1D e, f).

Electrolytes

The brain soaked in isosmolar (0.24 *M*) sucrose solution instead of Ringer's showed a progressive slowing of its rhythm and finally failed in 20 to 30 min. (Fig. 3A a, b, c, d, e). After 3 to 4 min. soaking, the 6 per sec. waves slowed sharply to 4 per sec. and increased 50 per cent in amplitude (*e.g.*, from 120 to 180 μV.). After another 5 min., frequency fell to 3 per sec., and an increased inter-electrode resistance indicated fairly complete loss of salts from the brain's conducting fluids. After 12 to 15 min. total soaking, the frequency again dropped sharply, to 1 per sec., irregular waves at 15 to 20 per sec. were often superposed, and the amplitude became less. The negative limb of these slow waves was longer than the positive and the high-frequency discharges occurred on the slight plateau of greatest negativity. Further soaking usually caused gradual loss of these potentials. In one case (with K and Ca still present; see below) the 1 per sec. waves were again supplanted by extremely regular rhythms at 4 per sec. Diphasic spike-like potentials of 200 μV. interrupted this regular sequence, occurring first in groups of 3, then in mixed triple and single groups, then singly, and finally disappearing (Fig. 3B f, g, h). The diphasic spikes are probably produced by traveling rather than stationary potential waves.

These changes were only partially reversible. Soaking the brain in Ringer's solution, after activity had disappeared in the non-electrolyte solution, led to feeble irregular potentials. Restoration to saline at the slow wave stage replaced these with faster less intense ones which lack the normal regularity. A solution containing sucrose in place of NaCl, but with the other ions of Ringer's solution present in normal amounts, affected potentials as does pure sucrose, except that about twice as much time was required for each change and the slow waves did not become so extremely regular (Fig. 3B). Increasing the K-ion (to 0.004 *M*) partially antagonized the effects of sodium lack, and led to potentials much like those in Ringer's solution following sucrose (Fig. 3A f, g, h).

Sodium lack was mainly responsible for the changes seen in non-electrolyte solutions. Addition to or subtraction from Ringer's solution of NaCl

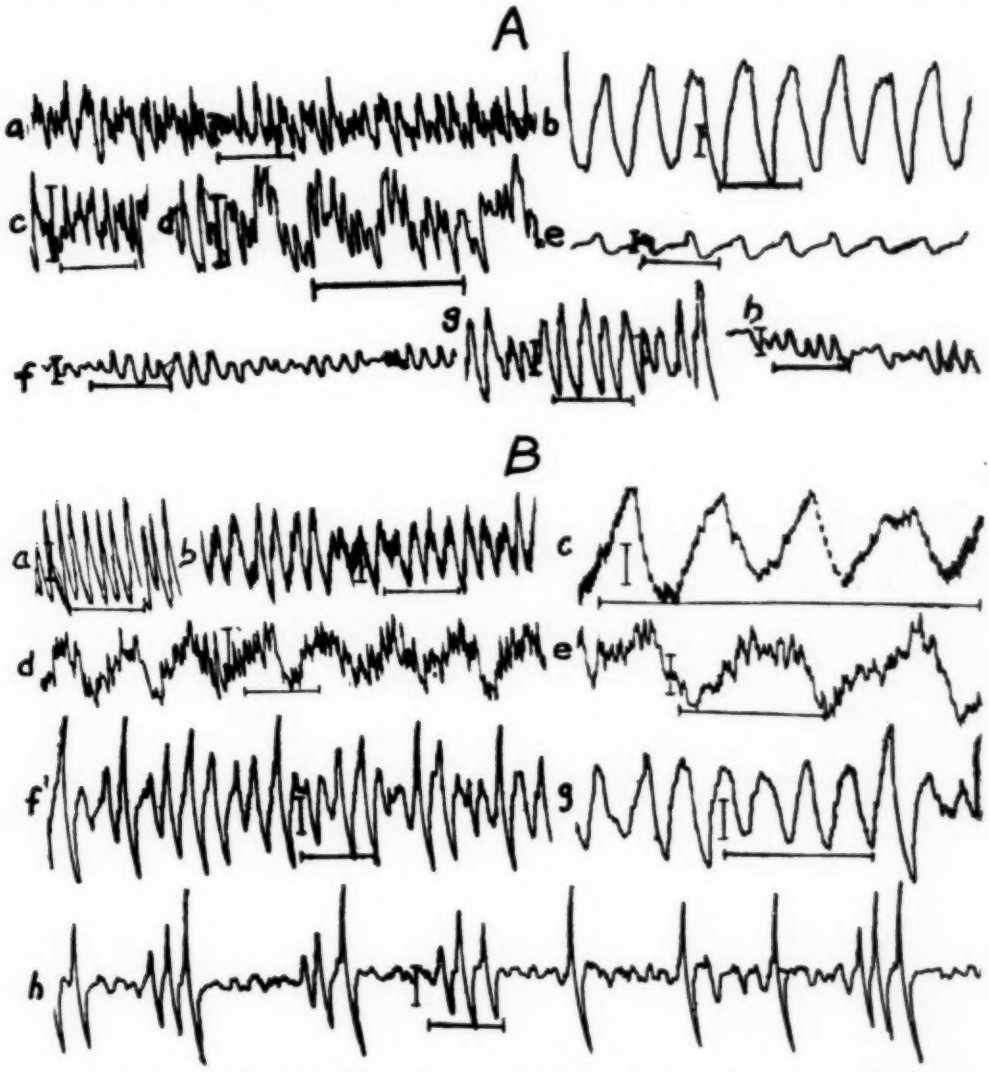

FIG. 3. A. a. Normal in Ringer. b. after 4 min. in 0.25 M sucrose. c. and d. 3 min. after return to Ringer. e. 8 min. after replacing in sucrose. f. Another brain in Ringer's after 6 min. in sucrose. g. 2 min. after return to sucrose. h. 3 min. after replacing sucrose with sucrose plus normal $CaCl_2$ and double KCl.

B. a. Normal in Ringer. b. and c. after 10 min. in Ringer with sucrose replacing NaCl. d. and e. after 20 min. in same solution. f. and g. after 30 min. h. 20 sec. after g.

in moderate concentration, *e.g.*, 0.02 *M*, did not significantly affect potentials. Doubling Na-ion produced feeble irregular rapid potentials, though the increased osmotic pressure, *per se*, would act in the opposite direction. Replacing half the Na-ion of Ringer's solution with the isosmotic equivalent

of sucrose slowed the waves about 20 per cent after 3 min. soaking, 30 per cent after 10 min., increased their amplitude 30 to 40 per cent (*e.g.*, from 110 to 150 μV.), and increased regularity.

Potassium acted much like sodium, but much smaller quantities were effective. Isosmotic (0.125 *M*) KCl initiated rapid feeble waves which faded completely after soaking for 4 min. In 0.01 *M* concentration, this salt led to

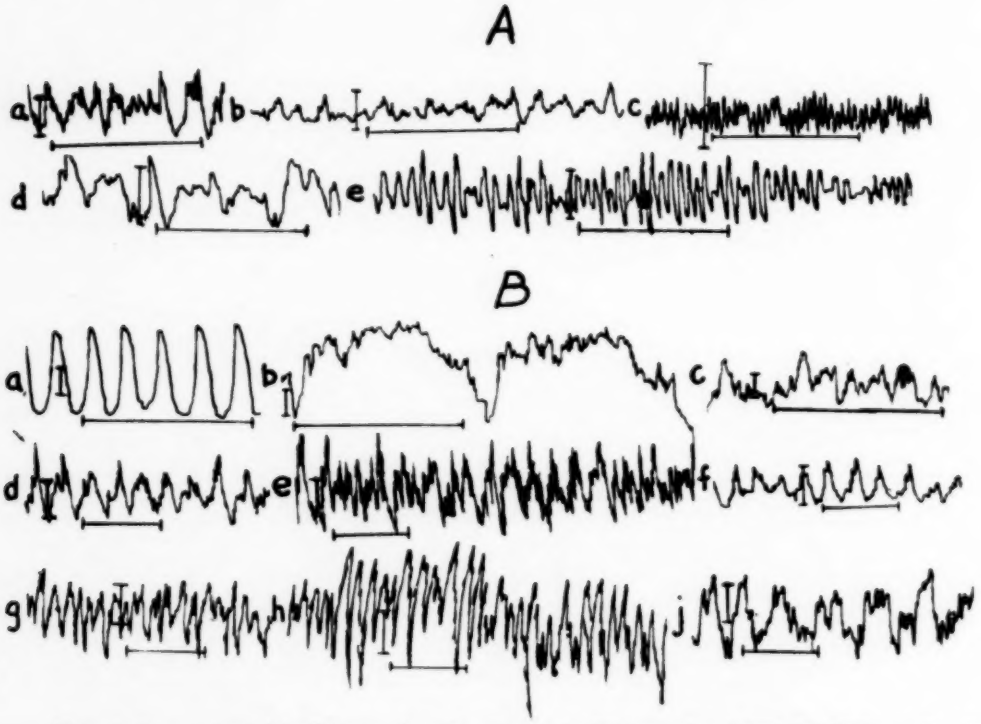

FIG. 4. A. a. normal in Ringer. b. after 3 min. in Ringer plus 0.003 *M* KCl. c. after 3 min. in Ringer plus 0.01 *M* KCl. d. another brain, normal. e. after 3 min. in Ringer plus 0.01 *M* KCl.

B. a. normal in Ringer. b. after 3 min. in Ringer plus 0.018 $CaCl_2$. c. 3 min. after return to Ringer. d. another brain, in Ringer plus 0.003 *M* $CaCl_2$. e. 3 min. after placing in Ringer plus 0.005 *M* Na citrate. f. 3 min. after return to Ringer plus 0.003 *M* $CaCl_2$. g. another brain, normal in Ringer. h. after 3 min. in Ringer plus 0.003 *M* $MgCl_2$. j. after 3 min. in Ringer plus 0.01 *M* $MgCl_2$.

irregular potentials in some cases, to regular waves at 20 to 30 per sec. in others (Fig. 4A). Concentrations between 0.003 and 0.01 *M* caused a sharp change from the normal to feeble somewhat irregular waves at 15 to 35 per sec. These changes were but little reversible when the brain was restored to Ringer. Weaker KCl, 0.002 *M*, however, gave a largely reversible increase in rate and decrease of amplitude (50 per cent) and regularity.

Calcium and magnesium (Fig. 4B) acted alike, and at similar concentrations, to change potentials in a manner opposite to that of sodium and potassium. Addition of 0.001 *M* $CaCl_2$ to Ringer's solution definitely slowed

the waves, and the effect was progressive with concentration to 0.007 *M*, which lowered frequency by 35 to 45 per cent (*e.g.*, from 7 to 4 per sec.). Amplitude was not altered and regularity perhaps improved. Larger Ca-ion additions, 0.018 *M*, caused a discontinuous further slowing to 1 per sec. waves of the same amplitude with feeble irregular potentials at 10 to 20 per sec. superposed. Return to Ringer's solution restored more rapid waves, but they never regained the normal regularity. Removal of calcium affected potentials as did addition of potassium. Sodium *citrate* (0.004 *M*) in Ringer's solution produced irregular rapid waves, and the normal slow rhythm was again restored by Ringer's solution with added $CaCl_2$ (0.003 *M*) (Fig. 4B d, e, f). Sodium sulphate acted like citrate but greatly depressed amplitude as well (*e.g.*, from 35 to 5 μV), and its action was irreversible.

Ion antagonism was apparent from the above results and clearly showed when potassium and calcium, especially, were balanced against one another. When both were removed from Ringer's solution, potentials in the remaining NaCl continued quite normal for about 10 min.; when both were added in equimolar amounts, up to at least 0.005 *M* addition, potentials also continued normal for some time; and when the excess of either ion had changed potentials, a slight imbalance in favor of the other improved and hastened the restoration toward normal.

Anions had little effect on potentials, except as they removed calcium. (Sulphate probably has a specific action as well.) At pH 7.4, mono- or dihydrogen phosphate (up to 0.013 *M* concentration) and bicarbonate (up to 0.02 *M*) were inactive. Substitution of half the chloride ion in Ringer's solution by iodide ion was without effect, and complete interchange of the two produced only minor differences. (Peculiar diphasic waves appeared at intervals in iodide.)

Discussion

A single olfactory bulb can be made to vary its regular rhythm over a wide frequency range by appropriate change in environmental conditions. All rates have been encountered between 1 and 10 per sec., the values 13 and 17 appear and, less sharply, rates of 20 to 25, 35 to 40, and 45 to 55 (Fig. 5). Amplitude tends to decrease with increasing frequency, as does regularity, but large or small waves have been seen at all rates. Further, the shape of the individual regularly repeated waves can be deliberately changed from an approximate sine form to a variety of highly unsymmetric profiles (Fig. 5).

The relatively homogeneous cells of the bulb, probably only a few thousand separate units altogether, can thus manifest potentials of a fixed regularity and even a fixed amplitude over a fifty-fold range in frequency and a wide array of wave shapes. These facts speak strongly for the existence of individual neurone potentials of frequency and form identical with those of the recorded potential. This latter is the integral of the unit potentials and will reproduce them, except in amplitude, only if all units are similar and in synchrony. With asynchrony of units no regular pattern could be recorded unless each cell became active in a constantly repeated time se-

quence, as in the case of the flashing lights of a sign which are engaged in order by a rotating contact drum. The reverberating circuits postulated for the brain (Lorente de Nó, 1938) might afford just the neural mechanism required for such a delicate timing; but they are unfortunately ruled out by experiments with nicotine (Libet and Gerard, 1938; Schweitzer and Wright,

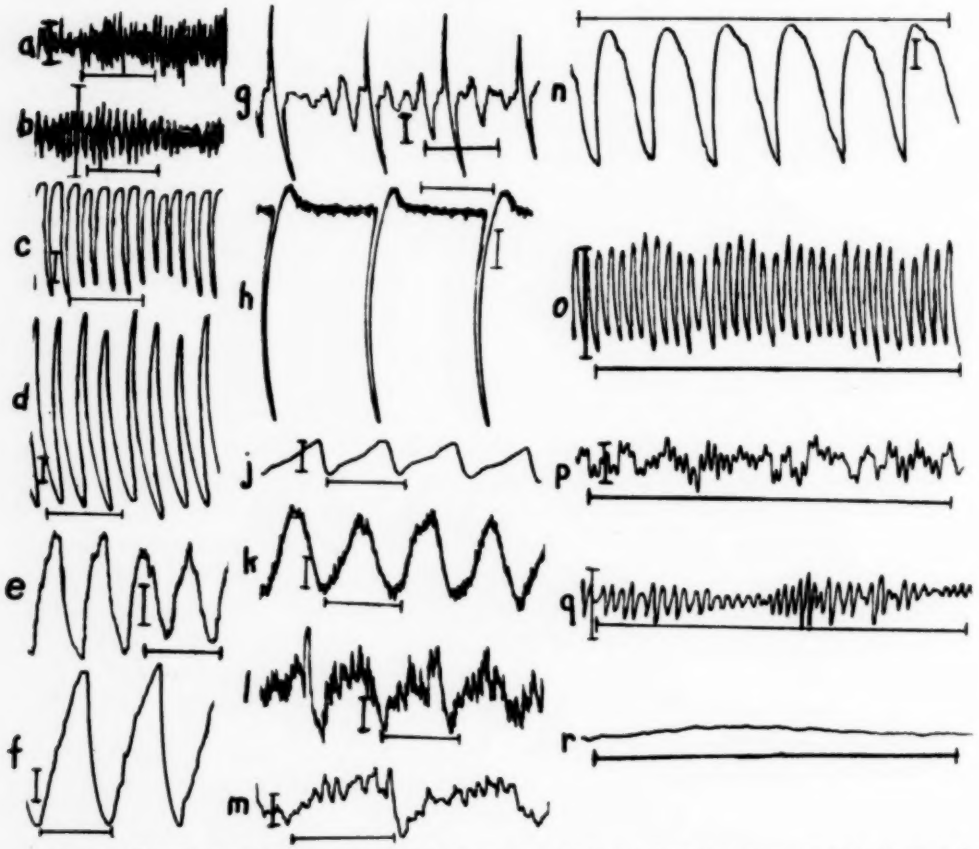

FIG. 5. Samples of rhythms obtained from isolated olfactory bulbs. a. freshly isolated, aberrant. b. 2 hr. in serum. c. freshly isolated. d. 3 min. in nicotine. e. 3 min. in sucrose. f. 6 min. in nicotine. g. 30 min. in Ringer with sucrose in place of NaCl. h. 3 hr. in stale serum. j. 10 min. in nicotine. k. 4 min. in nicotine. l. 15 min. in Ringer with sucrose in place of NaCl. m. 20 min. in Ringer with sucrose in place of NaCl. n. freshly isolated. o. small isolated bit of bulb. p. 30 min. in Ringer, aberrant. q. single separated bulb. r. control on optic lobe.

1938), which blocks synaptic conduction but leaves perfectly regular odd-shaped waves.

The range of frequencies exhibited by cells of one or few anatomical types (Gerard and Young, 1937; Adrian, 1937) must, then, represent a range of frequencies for each unit (see Blake and Gerard, 1937) or a regular sequence of action of varying numbers of cell groups. To the extent that doubling of frequency is accompanied by halving of amplitude, such an

alternation would be possible. But, though this is common, there are plenty of instances of a frequency increase with constant amplitude. Further, frequency may change smoothly with no discontinuities, or with jumps to values that are far from simple multiples of the initial ones. And, finally, any such beating in fractional groups would not account for a change to skew wave-forms, as produced by sucrose, nicotine, etc. The simplest hypothesis again seems to be that many single units beat in synchrony and so give a magnified record of their individual identical frequencies. This also fits with the fact that regular waves are obtainable from small fragments of teased olfactory bulb. It may be also, as Adrian (1937) suggests, that synchronizing occurs best at certain cell frequencies.

Changes in rate and form and, within limits, in amplitude induced by applied agents in *regular* potential waves would, then, be interpreted in terms of the individual neurone beat. But changes in regularity also occur—from an increased variability of successive distinct waves, through "fuzzy" waves with superposed potentials, and irregular alternations, to highly random potential swings—and these are best interpreted in terms of the mechanism of synchronization. Slight cell asynchrony will cause more apparent irregularity in short fast waves than in long slow ones; yet increase in temperature, which accelerates the rhythm, also improves its regularity. This is an especially clear case for a synchronizing mechanism, independent of the individual cell, which increases in efficiency with temperature. We shall, therefore, interpret the action of experimental procedures on potentials in terms of the unit neurone beat and the degree of synchronization of units.

Each cell exhibits a potential wave with amplitude, wave-length and shape characteristic for the extant conditions. The relation between frequency and intensity is especially important since, by physical analogy, at constant energy (from cell metabolism) the two should vary inversely. A change of rate without a change in amplitude, still more a parallel increase or decrease in both together—as in the case of altered temperature—must, then, indicate a change in available metabolic energy. (With improving synchrony, of course, amplitude could increase in the total record and not in the unit. This can often be excluded.) On the other hand, increased frequency with decreased amplitude might represent a change in the "setting" of some "trip" mechanism, with no change in the original metabolic sequences. The first case above would correspond to a change in the tension of a metronome spring, the second to a shift in position of the ballast on the pointer. Frequency variation due to alterations in metabolic rate would almost surely be smoothly continuous and essentially reversible, as with temperature; but variation due to resetting of the trip mechanism (some membrane property?) might easily be discontinuous and imperfectly reversible, as with altered ionic environment. Many agents unquestionably have a mixed action; and we shall report in a later paper studies, of the influence on these potentials of a number of metabolic inhibitors, accelerators, and other effective drugs, that help to clarify the cellular mechanisms which determine the electrical beat.

Little can be said as yet about the mechanisms which integrate and synchronize the beats of individual cells, but that such exist is certain. The findings with nicotine, etc., show that, though the passage of nerve impulses along conducting paths may play an important role, this is not a necessary or indeed the most important means of coordination. It has been suggested (Gerard, Marshall and Saul, 1936) that steady potential fields and polarization might contribute to synchronization, and a small constant current does increase cat brain rhythms as if improving cell unison (Dubner and Gerard, 1939). This experiment has not yet been performed on the frog brain, but some similar evidence is available from waxing and waning of waves.

Regular wave trains do not wax and wane but as the single waves become less constant this spindling is likely to appear. An interpretation would be that the cells are locked together by a strong synchronizing mechanism to give regular beats and that as this control is lessened the cells are more easily desynchronized, with first some fuzziness in the recorded potentials and then the coming in and out of phase which produces amplitude beats. There are indications that a steady potential of the brain mass is more negative during the large waves at the belly of a spindle and less so when the feeble waves occur at the ends of one. A greater constant potential is, therefore, associated with greater cell unison. That regular waves document a strong action of the synchronizing mechanism is further shown by attempts to disrupt them. It is constantly found that an agent which disturbs a steady rhythm, for example K ion, is effective in lower intensity with less regular waves. A very smooth beat persists in KCl concentrations which break up a less constant one and is disrupted only by greater concentrations.

These effects are seen with the passage of time *in vitro* and it is likely that one factor in the disintegration of the potentials of an isolated brain is the gradual failure of the synchronizing mechanism. The effects of stimulation might be partly via an improvement of this; and the fact that further isolation of a small bit of brain from a larger inactive mass brings out again a potential beat could also be explained in terms of leading from a few synchronized units rather than from many desynchronized ones. Finally, the improved synchrony induced by rise in temperature and by certain stimuli and by nicotine, sucrose or calcium, and the diminished synchrony with time of isolation, cold, potassium, etc., show that the synchronizing mechanism is a real entity with measurable properties permitting its further analysis.

It remains to note some impressive parallels between the behavior of brain and nerve. Brain rhythms have been compared to the oscillating after-potentials of nerve (Gerard, 1936; Gasser, 1937). In mammalian nerve (Lehmann, 1937a and 1937b; less completely in frog nerve, Graham, 1933) the after-potential rhythm is increased and the "wave-length" shortened by raised potassium or by lowered calcium or hydrogen ion. The reverse ion changes, although augmenting the first negative after-potential, lengthen the oscillation period and may completely depress the waves. These effects are entirely comparable to those produced in the frog's olfactory bulb (and

cat's thalamus Dubner and Gerard, 1939), although the ions act on the brain more rapidly and in lower concentration. (Compare Dusser de Barenne, McCulloch, and Nims, 1937, on the influence of pH on cortical potentials.) That the frog brain should be relatively insensitive to pH changes is not surprising in view of the fact that the pH of the frog's blood normally varies rather widely (see Rohde, 1920); also frog nerve metabolism is fairly insensitive to pH change (Gerard, 1930). With after-negativity of nerve goes heightened irritability and even spontaneous discharge (Gasser and Grundfest, 1936; Lehmann, 1937a); with positivity, depression. The same is true for grey tissue (Adrian, 1931 and 1937; Hughes and Gasser, 1934; Eccles, 1936; Heinbecker, 1936; Barron and Matthews, 1938); and, in both, potassium and calcium powerfully control potentials, activity, and after-discharge (Gerard and Magoun, 1936). Further, in both brain and nerve there are similar powerful cation antagonisms while anions are relatively unimportant. Spike height in nerve is reduced by excess of either potassium or calcium (Graham, 1933) and the same is true in brain cortex (Dubner and Gerard, 1939).

Finally, nerve after-potentials are minimal in the well-rested tissue and increase with repeated activity. Their persistence, amplitude and oscillations, following a single impulse, depend on previous excitation and contemporary physico-chemical environment. The brain potential oscillations similarly are influenced by past activity as well as current environment; stimulation enhances the wave for a considerable time afterward and the usual rhythm fades out over hours in the unstimulated isolated bulb but can be restored for many minutes by a brief barrage of impulses. A like interpretation seems to fit the sequence of potential changes of the human cortex in sleep (Blake and Gerard, 1937; Blake *et al.*, 1939). It is also not improbable that the increased frequency commonly observed when a bit of brain is separated from a larger piece is due to injury potentials at torn processes, etc. It will be desirable to compare the behavior of such brain rhythms with that of the demarcation potentials of nerve and brain with change in time, temperature, ion concentration and the like.

The action of ions on brain and nerve metabolism has been reviewed elsewhere (Gerard, 1932 and 1937) and here it need only be mentioned that for brain respiration, as for its potentials, sodium and potassium synergize in increasing it, with potassium far more active per mole, and calcium and magnesium depress it; while anions have little effect (*e.g.*, Dickens and Greville, 1935). Whether the persistence of a rhythm in a salt-free medium means that inorganic ions are not indispensable to the potential control or that these are incompletely removed even from the cell exterior, cannot be answered from these results. At least the brain neurones have a sufficient store of substrate, and obtain adequate oxygen for the oxidative portion of their metabolism, to maintain their beat in isolation. Their ultimate failure is due only in part to loss of background excitation and must finally depend

on depletion of reserves or on disintegration of organization in the abnormal *in vitro* environment.

Summary

The olfactory bulb of the isolated frog brain, which continues its *in vivo* electrical activity (especially a large regular 6 per sec. rhythm), was used to investigate physico-chemical and nervous factors controlling such spontaneous potentials.

Wave size and regularity are enhanced immediately upon isolation, without change in frequency, then gradually decrease to zero during about 3 hr. in Ringer's solution and 4 in serum. Wave frequency usually increases with each step of further isolation (single bulb, bit of bulb), and regularity, if anything, is improved.

Electrical stimulation can restore some activity of the "run down" brain; and, even in the freshly isolated one, stimulation of the olfactory nerve for seconds increases bulb potentials by 25 per cent for 10 min. following.

Rise in temperature, between 5 and 30°C., increases frequency (the average Q_{10} is 2.3) and amplitude, and always improves regularity. Cooling has the reverse effect.

Doubled osmotic pressure, radically reduced Na-ion, moderately increased Ca- or Mg-ions, or lowered pH produce slow waves; while increased K, Na, or pH and reduced Ca produce fast ones. Na and K are antagonistic to Ca, K in small concentrations being more effective than Na. Effects are generally progressive with concentration. Changes in frequency are usually discontinuous, and when extreme are irreversible. Anions are generally without marked effects.

Although originating in a small homogeneous neurone population, potential patterns may vary greatly in frequency, wave shape, and regularity depending on the factors studied. These and other related facts are discussed in relation to the problems of frequency and amplitude of single neurone rhythms and of the mechanism coordinating them to give the recorded potential. Significant parallels appear between rhythmic nerve potentials and those of cerebral neurones.

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BRAIN WAVE FREQUENCIES AND CELLULAR METABOLISM. EFFECTS OF DINITROPHENOL*

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A VARIETY OF EXPERIMENTS indicate that the relative *frequency* of brain potentials, other things being equal, depends on the rate of respiration of the cells producing the rhythm. The temperature characteristics of alpha frequencies in persons subjected to diathermy coincide quantitatively with the major modal values encountered for cellular respiration *in vitro* (Hoagland, 1936a). Lowering of blood sugar by insulin, which results in subsequent lowering of brain sugar, causes alpha frequencies to diminish in rate, but with a lag. Injection of sugar restores the frequencies to normal (Hoagland, Rubin and Cameron, 1937; confirmed by Himwich *et al.*, unpublished). Sugar is probably the only fuel used by the brain (Himwich and Nahum, 1932). Thyroxin accelerates alpha frequencies (Rubin, Cohen and Hoagland, 1937), and nembutal, known to inhibit cellular respiration (cf. Page, 1937), slows the rhythms (Hoagland *et al.*, 1939). Himwich, Hadidian and Fazekas (unpublished) have demonstrated a relation between O_2 consumption of the brain and alpha frequencies, and Hoagland (1936b) has discussed physical models which would produce electrical rhythms at frequencies proportional to continuous chemical events of the type under consideration.

In view of these findings one might expect that dinitrophenol (an effective metabolic stimulant in respiring cell systems *in vitro*, as well as in intact mammals; cf. Dodds, 1934) should produce acceleration of brain wave frequencies.

METHODS

Electroencephalograms have been recorded on paper tape by two ink-writing undulators and matched amplifiers built by Albert Grass. The electrodes were wires attached to lead pellets 2 to 3 mm. in diameter, the pellets making contact with the scalp through electrode paste and held in position by collodion. One grid electrode was placed over the occiput, 2 cm. above the inion and the other was placed over the vertex. The indifferent lead was attached to the skin behind each ear and parallel leads from these attachments were brought together to form a common connection. Tests showed this ground lead to be electrically inactive. Our tape speed was 30 mm. per sec., our amplification 10 μV. = 1.5 mm., and our time constant 0.2 sec. Four persons were selected who showed dominant, clear, alpha rhythms at *both* occiput and vertex. These subjects were male schizophrenic patients who were more conveniently available for our tests than normal persons would be who might show equally dominant and, therefore, countable alpha waves at both vertex and occiput. There was nothing about the electroencephalograms of these patients that would characterize them as abnormal. As in a number of persons we have examined, the

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alpha frequency from the vertex was uniformly about one cycle per sec. slower than that from the occiput. The simultaneously paired records showed electrical independence of each other, as would be expected (cf. Rubin, 1938).

Records were made on the subjects while they were reclining with closed eyes. On the first experimental day, prior to medication, about 5 min. of continuous records were taken, after which, at 1:30 p.m., each subject was given by mouth doses of 4.0 mg. per kg. body weight of 2, 4 dinitrophenol, and at intervals throughout the afternoon other 5-minute samples of electroencephalograms were made. On each of the 4 following days the same dose of dinitrophenol was administered at 1:30 and records were obtained 2 hr. later. The clear alpha frequencies were counted from 100 sec. samples (*ca.* 1000 alpha waves per sample) and their mean frequencies per sec. determined for purposes of plotting.

Results

Figures 1 and 2 show the results obtained with two of the subjects which, in general, are typical of the four. Frequencies from both vertex (lower

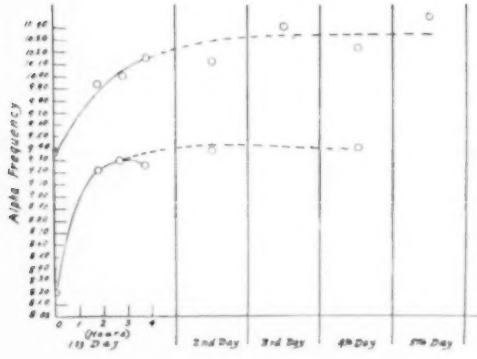

FIG. 1 (see text)

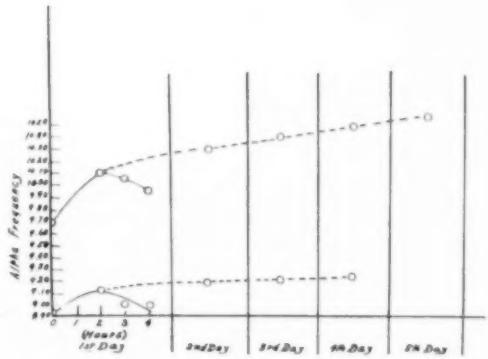

FIG. 2 (see text)

curves) and occiput (upper curves) are accelerated the first 2 or 3 hr. on the first day. Later the dinitrophenol effect apparently wears off and the frequencies tend to fall (solid curves). However, some residual effect remains since the same dose given at 1:30 on subsequent days gives for readings 2 hr. later a steady, slow rise in frequencies (dashed curves). On the 5th day alpha waves from the vertex in these 2 cases were irregular and no longer countable, though occipital alphas were as dominant as ever. The alpha waves from the vextex in the other 2 patients remained clear on the 5th day but the data throughout were somewhat more variable. Hoagland (1936a) showed that the probable errors of alpha frequencies counted in this way involving comparable data are of the order of ± 0.05 cycles per sec. This is about the diameter of the circles making up the plotted points. The curves are, therefore, highly reliable statistically. Data obtained 4 days after the termination of dinitrophenol medication showed that all of the frequencies were still abnormally high. A week later, however, they were back to the normal preexperimental level.

These experiments make it clear that dinitrophenol accelerates cortical brain wave frequencies in a way to be expected of this specific respiratory

stimulant, if our premise at the beginning of this paper is correct. It is especially interesting that rhythms from both occiput and vertex are independently accelerated in each person to approximately the same degree and along similar curves. Individual differences of the subjects are also suggestive.

Summary

1. Occipital alpha brain wave frequencies and occipitally independent alpha type waves from the vertex, which are about one cycle per sec. slower, were studied simultaneously with independent double recording systems in 4 subjects before and after doses of dinitrophenol.

2. The independent rhythms from both regions are accelerated along two smooth curves in a manner to conform with the view that the frequencies, under the conditions of these experiments, are a measure of cortical respiration.

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